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Medical policymaking in Britain

If there is any subject that is guaranteed to generate boredom amongst scientists it is science policy. The average practitioner believes, with some justification, that whereas it would be most undesirable for a country to eschew a defence policy and leave all decisions, from procurement to strategy, to the soldier in the field, the scientist should be immune from policy constraints.

This system, in which the majority of scientists have received the majority of what they asked for and have in general been answerable only to their own consciences and to a relatively genial sponsor, is beginning to crumble as the growth rates for expenditure on research drop dramatically and as the demand for accountability rises sharply. Within the next year or two many scientists will indeed discover that there is such a subject as science policy, and arid and irrelevant as it may have seemed in the past, it is science policy that will decide whether their project will continue to be funded. The recent publication of the Medical Research Council's Annual Report (HMSO, 97p) is an appropriate time to look at the policymaking machinery in the biomedical field, and the report is generous in its attention to policy. The council is still able to say that "the ideas and interests of the individual scientists at the bench are the driving force behind science", although it adds in the next sentence "there may also need to be active discouragement of over-populated but low priority areas"—a fairly even-handed way of mixing good and bad news.

And yet there is something strangely and almost intangibly missing from the report. It is most easily seen in the section describing progress in research on selected topics. One gets the unmistakable impression that the Medical Research Council is the last bastion the world has against disease and death. The report is of course addressed primarily to the Secretary of State for Education and Science (who is told in the second paragraph of the transmittal that for £1.00 he can buy the council's Handbook from headquarters), and as such necessarily must remark on the successes that government money has bought. Nevertheless there remains just the vaguest feeling throughout the policy section that this is policy for medical research determined by the Medical Research Council without mention of the fact that a quite substantial amount of research is done by other research councils, by charities and trust funds, and by the pharmaceutical industry. Of course, excellent liaison may exist, although it doesn't shine through. And it is obviously desirable to allow a multiplicity of funding sources to exist so that power does not reside solely in one council, however appointed. But if it is indeed the function of the MRC to promote the balanced development of medical and related biological research and if all research programmes are going to fall on harder times in the next year or two it would be good to know that policymaking reflects the 'mixed economy' nature of Britain's support of medical science.

Dear Mr Turner . . .

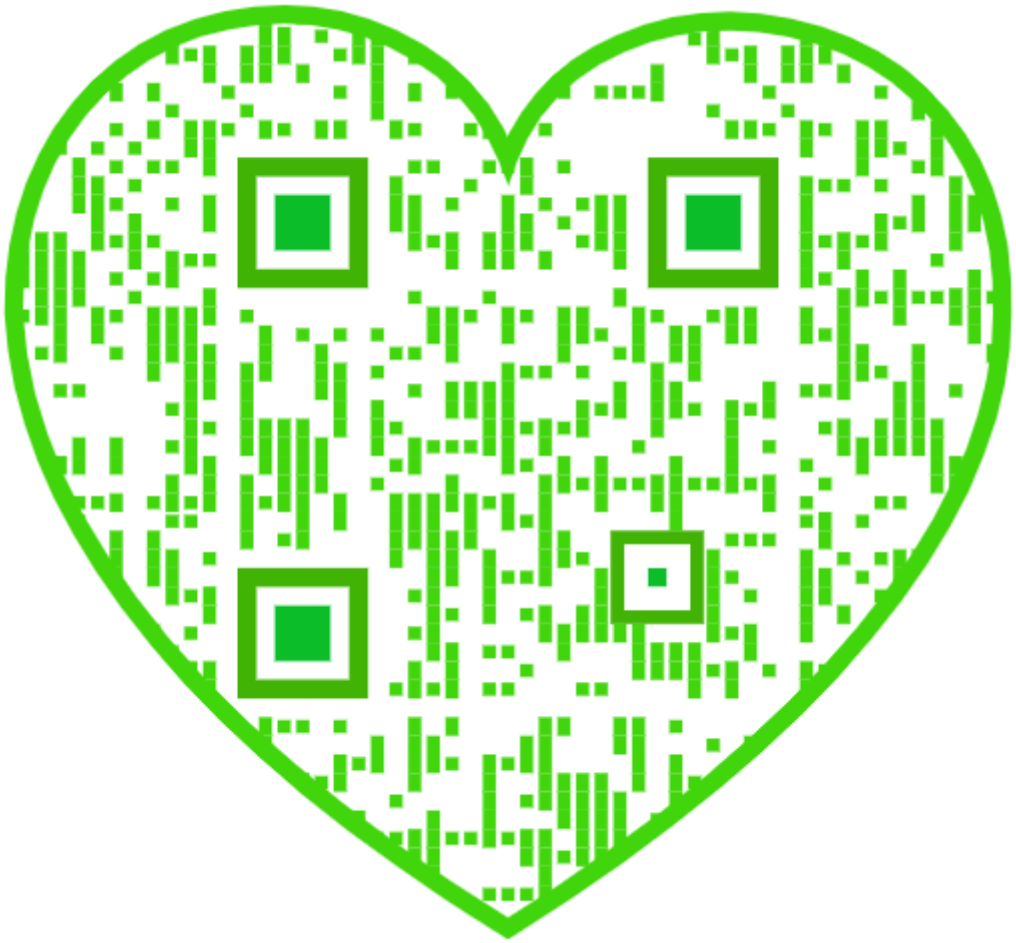
By August 1 you had hoped to have appointed several research fellows to pursue fundamental biological research. Instead, as everyone now knows, you came into conflict during the selection procedure with potential recipients of the awards who objected to your refusal to consider those who had religious beliefs of one kind or another. No doubt you feel sore at all this and believe that there are fundamental inconsistencies between religion and science which cannot be glossed over by research scientists.

You are by no means alone in your doubts that the pursuits of science and religion are incompatible occupations; indeed I suspect that amongst businessmen it may be a majority view. Of course, there are many scientists whom you would probably happily exclude from your strictures—those working in agriculture, medicine, materials and so on are presumably exempt, but you would probably wish to retain those subjects which move close to the problems of man and the universe, such as astronomy, geology and those branches of biology that are concerned with the nature of life. How, you might say, could someone dare to contemplate an idea in these fields which struck at his own personal convictions.

As you know, there have been scientists of great distinction who have held very strongly to religion and, one suspects, many more who have believed in some underlying purpose to the universe without feeling that they had to devote an hour to it every Sunday. Perhaps some were inhibited from peeping too far behind the scenes, although I know of none, but many more were positively driven on by their beliefs to understand more about a universe at which they marvelled.

Furthermore successful scientists learn flexibility early in their training—they cannot afford to become too committed to conventional wisdom because they are there to discover, not to reinforce. Thus they are unlikely to hold fast to any religious beliefs with which they know their research might bring them into conflict. They are also pragmatic and like fame as much as anyone else, so they are not going to stand by for the sake of religious beliefs whilst someone else finds a cure for cancer or a recipe for longevity.

Most scientists would have found it extraordinarily difficult from their personal experiences in the fields I have mentioned to categorise scientists as religious or otherwise from their mode of working and their approach to fundamental questions. You would probably have found the same.



Call for biohazard legislation

In this article Brian J. Ford argues that the law does not concern itself sufficiently with the various hazards that can arise when microorganisms are mishandled and suggests areas in which legislation should be introduced.

LEGAL restraints and regulations are the bane of research workers in many spheres. All of us must have felt some kind of annoyance at the filling in of forms or the signing of registers that have the aura of state bureaucracy, but the justification lies in the resulting safety of research. If that is the case, it is odd that legislation covering the handling and use of microorganisms and viruses that are pathogenic to mankind is virtually nonexistent.

At the moment moves are afoot in Britain to make hepatitis a recognised industrial disease amongst hospital and laboratory staff, and there is in the United States a call for a moratorium on microbial genetic manipulation. Such piecemeal short term measures could be superseded by the greater controls of a generally accepted legal framework of safety.

In other fields of scientific endeavour there are laws that regularise the use of potentially dangerous materials. Strict controls govern the use and distribution of radioactive substances, even those of the lowest potency used in student demonstrations. Toxic chemicals are subject to a range of long standing controls including the pharmacy and poisons legislation, the poisons rules and the legal restraints on dangerous drugs. In the First Schedule of poisons appear compounds great and small, from TEPP to 2-methoxycarbonyl-1-methylvinyl-diethyl phosphate; yet there is no reference to the bacterial toxins which are considerably more dangerous and also, in many respects, easier to produce.

In the regulations governing the security of the experimental animal appear rats, mice, rabbits; even dogs and cats. There is no mention, however, of pathogens. In the world of agriculture there are restrictions on the handling of some plant pathogens of economic significance and on the culture of viruses such as foot-and-mouth, though limitations on human pathogens are absent.

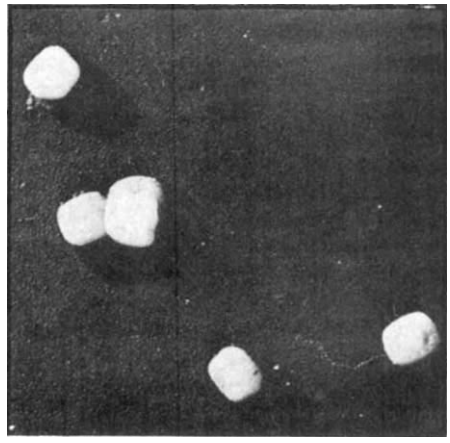
In philosophical terms the greatest hazard posed by the lack of controls over pathogens is their infectivity: poisons do not replicate. A technician who takes home an isotope may damage himself as a result, whereas a mishandled culture of virulent microorganisms could expose entire communities to widespread hazard. Yet isotopes are kept under lock and key. It is a culture of pathogenic microorganisms that needs no such safeguard in the eyes of the law.

Since I first published an outline of proposals for biohazard legislation (*New Law Journal*, **121** (5511), 823–824; 1971) there have been several examples of the misuse or abuse of cultures pathogenic to mankind. That paper referred to the virus of smallpox (variola) and the occasional accidents that occur when unsupervised or inexperienced staff come into contact with pathogens. Last year's outbreak in the London area is a tragic illustration of the hazard.

Children have recently been found playing with discarded culture vessels in the Midlands; and others were found to have obtained contaminated syringes casually discarded by a pharmaceutical concern in Kent. Occasionally cultures are wilfully misused. In the United States it has been reported that an underground group planned to immunise themselves against the effects of bacteria that were then to be introduced into the public water supply, and in Australia a culture of a meningitis virus was stolen (in its incubator) from a research laboratory.

Examples are known where disease has resulted from the use of unplugged mouth pipettes. A recent example in Britain involved a supposedly non-pathogenic bacterium ingested when the tip of a pipette under suction was momentarily withdrawn from a thin agar culture. Elsewhere an assistant handling positive sera from syphilitics made a similar error, but did not develop the disease. I have seen a technician attempt to open a phial of polyvalent poliovirus, believing the glass to be scored. It was not, and the neck fractured and lacerated his finger.

Smallpox virus particles



It had not been ascertained whether he was immune to polio before commencing his work with the viruses. A similar case concerned a girl technician who saw her doctor with a sore throat, contracted shortly after she began to work with diphtheria. No check had been made on her immunological status and following representations made by the doctor to the college department the entire research team was immunised as a precaution. At a university zoology department, to cite another example, staff set up a tank for the rearing of water snails infected with bilharzia. A small child related to one of the staff dabbled its hand in the water on the morning before the parasites were introduced.

In each of these episodes some safety measures were subsequently introduced to prevent a recurrence. But retrospective, custom-built regulations are no way to prevent accidents entirely. We now have a situation where safety codes in a laboratory tend to be based on the past history of accidents in the establishment. Biohazard law would enable general safety measures to be introduced and enforced for the protection of all.

The literature of the past five years has included several examples where widespread hazards might have been involved. A form of infectious choriomeningitis reportedly caused five cases with three fatalities during experiments in the United States, and an unidentified virus from vervet monkeys has been stated to have caused over 30 cases with seven deaths despite the most rigorous attempts to contain the virus. Venezualan equine encephalitis is now said to be epizootic in Utah, having originated in biological warfare experiments.

Many hazards arise from the failure to maintain adequate supervision in laboratories. Junior or untrained staff may be given access to culture materials they do not understand. In some cases infected materials are disposed of by being poured down the same sink as



The revealing lens, 19th century version. A William Heath etching of the 1820's depicts the contemporary view of microbiological hazards

that used for the preparation of tea and coffee.

There are interesting precedents in British law for the kind of proposals I envisage. The Factories Act 1961, Section 64, provides for the minister to extend the provisions banning eating in places where fumes or dangerous industrial dusts are produced; there is no mention of the far greater hazards that have biological origins (and, as the important case of Weston *v* LCC (1941) showed, a technical institute is not a factory).

The conduct of workers in laboratories has an interesting precedent in the Agriculture (Poisonous Substances) Act 1952. Section 1 Subsection (c) calls for the "abstention from eating, drinking and smoking in circumstances involving the risk of poisoning" and Section 2 Subsection 1 (b) makes it a culpable offence to do anything to "wilfully . . . cause risk of poisoning". Similar precautions with respect to microorganisms and viruses would not be hard to define.

The Radioactive Substances Acts, 1948 and 1960, define in detail the conditions under which radioactive substances should be handled, distributed, stored and controlled. The clear restriction of the availability of radioactive materials to "duly qualified medical practitioners or registered dental practitioners" is similar to what might be proposed for virulent pathogens. The Factories Act 1961 Section 18 Subsection (2) refers to the avoidance of dangers from "scalding, corrosive or poisonous" materials, to which list the infection risk could be appended. Section 82 of the same Act contains an anomalous, isolated reference to bacterial hazards when it refers to illnesses caused by lead, phosphorous, arsenic, mercury "and anthrax" as notifiable conditions, without reference to other infections of an occupational origin. The widespread use of microorganisms in industry, which an extention of biological engineering will inevitably entail, makes safeguards timely.

Conditions of storage have been covered in respect of phials containing vaccines, antibiotics and related biological products under Part III Section 5 (i) of the Therapeutic Substances Act 1956, which states that such containers must be sealed to "preclude the access of microorganisms" and later specifies the labelling of the outsides of the containers. A universal biohazard mark for glassware and other apparatus used to contain pathogens should be found. Perhaps a yellow spot or disk could be fused into glassware during manufacture as a permanent warning against the casual use of beakers or flasks for the preparation of refreshments. The recently introduced biohazard symbol displayed in some laboratories, or something like it modelled on the radiation sign, should be obligatory in all establishments handling pathogens.

Certain diseases are already notifiable, of course; though it is interesting that an organism in one's gall bladder may lead to restrictions by the health authorities which they would be powerless to impose if the organisms were carried, in a bottle, in one's trouser pocket instead. The only substantive reference to microorganisms occurs in the Therapeutic Substances Act Part II Section 4 (b) of 1956. It requires that separate laboratories be provided by licensees producing medical products if they "engage in the culture or manipulation of pathogenic spore-bearing microorganisms". It is already recognised by this Act that pathogenic contaminants must be kept out of containers of vaccines and the like, though the converse possibility—that organisms allowed to escape from within containers may pose even greater problems—is not referred to.

By far the greatest indictment of the current situation is the framework of controls within the Public Health Act 1936 and 1965; and the Health Services and Public Health Act 1968 (as amended by Schedule 14 and para 4 of Schedule 29 to the Local Government Act 1972).

These regulations contain a host of strictures on those unfortunate enough to contract smallpox, with limitations on the movement of people concerned and the use of library books and dustbins during an outbreak. The possibility that restrictions should be placed on the *in vitro* virus remains ignored.

Legislation should cover several specific areas:

- It should define groups of organisms, such as those of high pathogenicity (variola, tubercle, typhoid) which are not normally present in the environment but which pose problems to communities. These I have grouped as 'Schedule A' pathogens. The remainder, including streptococci and staphylococci, are widespread and should be subject to more basic safety regulations in the laboratory: these would constitute 'Schedule B' and would alone be available for school and teaching use.
- The status of licensed holders of Schedule A organisms and viruses would be defined in terms of academic training responsibility and seniority. Less severe restrictions would be placed on laboratories classified for the culture of Schedule B pathogens.
- Standards of safety in laboratories would be laid down and codes of conduct made mandatory. The registration of holders of specified pathogens would doubtless aid the coordination of the research effort, and clear sets of agreed criteria would apply to genetic manipulation of bacteria and viruses, and to the disposal of potentially hazardous materials.

It is indeed unfortunate that bacteria and viruses are still so widely seen as harmful. To many of them we owe our very existence. Yet they confer on the research worker considerable responsibility when he comes to work with pathogens *en masse*. A legal code is now overdue for academic, practical and humanitarian reasons. □

international news

Test ban treaty condemned

by Colin Norman, Washington

Two groups which have long been advocates of arms control and of detente between the United States and the Soviet Union last week condemned as "a counterproductive sham" the so-called test ban agreement which the beseiged Mr Nixon brought back from Moscow. Suggesting that the agreement will do more harm than good in preventing the spread of nuclear weapons, the two groups—the Federation of American Scientists (FAS) and the Arms Control Association—called on Nixon to renegotiate a more meaningful arms control measure instead of sending the Moscow test ban treaty to the Senate for ratification.

A so-called "threshold" ban, the agreement would prohibit the United States and the Soviet Union from testing nuclear weapons with a yield greater than 150 kton. It is not due to be brought into effect until March 31, 1976. But it does not cover so-called "peaceful" nuclear explosions and it is simply a bilateral agreement between the Soviet Union and the United States, with no provision for other countries to become parties to it.

The FAS and the Arms Control Association believe that the treaty will do nothing to limit the number of tests conducted by both countries and that it will be seen as "a complete and cynical fraud" by countries which do not now possess nuclear weapons and which are looking to the nuclear powers to show some restraint in weapons development. In short, they argue that the Moscow agreement is worse than nothing.

They base their condemnation of the measure on four chief grounds.

- The 150 kton threshold—which is ten times greater than the bomb which devastated Hiroshima—is so ludicrously high that it will allow the United States and the Soviet Union to continue testing virtually unchecked. Furthermore, since it will not be introduced for nearly two years, both countries will probably carry out what the Arms Control Association calls an "orgy of intensive nuclear testing" in order to get some large tests in before the deadline.
- There are at least two, and possibly three, weapons systems which will have to be tested before the limitation comes into effect. First, a new warhead which is being developed for the Minuteman III, with a yield of about 400 kton, will have to be extensively tested. Second, a new bomb or perhaps an air-launched missile will have to be developed for the B-1 bomber. And finally it has been suggested that the missile for the new Trident submarine may have a yield of 200 kton; that too will have to be developed before the treaty takes effect.
- As for the Soviet Union, the chief programme which will be affected will be the development of MIRVs for the big SS-18 missile. Although according to some accounts testing for that device may already be completed.
- Aside from these weapons, there is little need for either country to conduct large tests—indeed, since the partial test ban was signed in 1963 the vast majority of tests have been below 150 kton—and so the FAS believes that the treaty will have little effect on weapons development. Thus seen from the viewpoint of non-nuclear countries, the agreement is a sham which does nothing to fulfil the commitment enshrined in both the partial test ban treaty and in the nuclear nonproliferation treaty to bring about a complete halt to weapons testing.

Equally counterproductive as far as stopping the spread of nuclear weapons is concerned is the fact that the agreement does not cover explosions for peaceful purposes. Since India justified its recent nuclear test as a perfectly peaceful enterprise—an interpretation which has been greeted with great scepticism outside India—FAS and the Arms Control Association believe that the Moscow agreement will increase the chances of other countries following India's example. As Adrian Fisher, former deputy director of the Arms Control and Disarmament Agency, put it last week, the agreement "legitimises the Indian position" at the time when everybody is attacking it.

The FAS also points out that the test agreement may pose severe political problems because of difficulties in ensuring that it is not violated. For one thing the treaty provides for calibration shots to be carried out at each country's test site, but FAS notes that "we cannot know the yield of the Russian weapon from examination of seismological data for reconnaissance. It could be 300 kton instead of the stated 150 kton". And for another, the same explosive force can give different seismological signals of differing strength, depending on where and how it is fired.

Finally, and perhaps most important, there is considerable concern that if this essentially meaningless agreement is ratified it will, in the words of the FAS, "sell out the efforts to reach a comprehensive ban" on nuclear testing by "retreating on policy grounds and taking the matter off the national agenda".

Thus FAS and the Arms Control Association are suggesting that the treaty should be renegotiated, with the objective of securing a complete embargo on nuclear tests. In any case, it is worth pointing out that some 37 senators, led by Edward Kennedy, sent a letter to Secretary of State Henry Kissinger during negotiations on the Moscow agreement asking that a comprehensive rather than a threshold agreement be negotiated. If they all stand firm in their belief that the Moscow agreement is meaningless in terms of control, they have enough votes to block its ratification if it is referred to the Senate.

If the matter is renegotiated, what grounds are there for believing that the Soviet Union would be prepared to negotiate a complete test ban? Two statements of Chairman Brezhnev are being widely quoted as giving a hopeful indication. First, on June 14 he said that the Soviet Union "was prepared to reach agreement now with the United States on the limitation of underground nuclear tests, proceeding to their full termination according to a coordinated timetable". And second he said in a speech on July 21 that "we would like to achieve something more and are prepared to go further; the Soviet Union is ready in particular to conclude an agreement on complete cessation of all underground tests of nuclear weapons".

It is widely assumed, however, that it was the Soviet negotiators who were most insistent in exempting peaceful nuclear explosions from the Moscow agreement, and that it could present a huge problem in any future negotiations. As the FAS succinctly put it, "the question arises of either talking the Soviet government out of its interest in peaceful uses or deriving an acceptable method of verification".

Maplin project abandoned

FOLLOWING hard on the heels of long awaited decisions about British nuclear power and oil comes the not unexpected news that the Maplin project for a 'third' London airport is to be abandoned. The decision has been made in the light of a reappraisal of the project made since the British election earlier this year, and comes just after the publication of the British Airports Authority (BAA) *Annual Report 1973/74* (HMSO, £2).

In that report, the Chairman of the BAA, Nigel Foulkes, commented: "The financial year brought a series of events which reduced the BAA's profits, slowed down the growth rate of the air transport industry sharply, and put all long-term plans for the future development of our airports back into the melting pot". What has now emerged from that melting pot is evidence that no further main runways will be required to handle expected traffic at the four airports in the London area (Heathrow, Gatwick, Stansted and Luton) before 1990. With the noise nuisance now likely to be lower than forecast by the Roskill Commission (thanks to the advent of quiet wide-bodied jets) there is no need for a new airport at least until that time.

Capacity at both the principal London airports would have to be increased whether or not the Maplin project went ahead, the Secretary of State for Trade, Mr Peter Shore, told the House of Commons on July 17. The growth envisaged is from a capacity to handle 20 million passengers a year to 38 million in the case of Heathrow, and from the present 6 million passengers a year to 16 million for Gatwick.

If air traffic expands so that even this capacity is insufficient to meet the demand, various possibilities will be open to the planners of the mid-1980s. Stansted, which has been referred to as the "expansion chamber" of the existing British airport system, must once again seem high on the list for future development, and although Mr Shore commented in the House that major diversion of traffic from the south-east to airports beyond Bournemouth, Birmingham and the East Midlands does not seem attractive at present, in the long term there is still a possibility of developing airports as far away as Prestwick and Edinburgh. With the higher speed rail links which would be possible by the 1990s, such a diversion might then have more appeal.

Whatever happens in the long term, in the present atmosphere of uncertainty about air traffic and with general economic stringency, many will, no doubt, echo the words of Mr Eric Moonman, Member of Parliament for Basildon: "To save £650 million is a good afternoon's work".



Last of the great independents?

LORD ROTHSCHILD said in 1971, a year after the appointment of a Central Policy Review Staff (CPRS) or 'think tank', that its and his success could be measured in terms of its survival. It had then survived somewhat longer than the equally novel Department of Economic Affairs—a parallel he drew—and this was despite the unnecessarily disruptive rumpus caused by the blunt instrument of the White Paper on the reorganisation of government research funding. The think-tank's continuation has now been officially endorsed by the present Labour administration and so tacitly subsumed as a permanent piece of government machinery, but Lord Rothschild is opting out. His successor as head of the CPRS is Sir Kenneth Berrill, a Treasury economist and former Chairman of the University Grants Committee.

The virtue of the original think-tank (whatever its shortcomings) was that it was Lord Rothschild 'writ large'. The fifteen or so bright young men that formed its analytical staff were all of his personal choosing. He was not, nor ever could be, a conditioned civil servant. His independent means removed him from the servitude of security in presenting his opinions—though he has questioned the relevance of this. His background lay in demanding fields of observational science and experiment, his foreground in multinational industrial research planning in his 10 years as research director of Shell Chemicals. All the indications are that however strange the advice proffered by Lord Rothschild's think-tank it was genuinely independent. The change can only mean a reduction in the role of science in government, especially as the post of Chief Scientific Adviser has also lost status recently. Does the government really want informed, independent pacing of its performance 'within the system'?

Two years ago Lord Rothschild assessed the importance of the CPRS as follows: "Regional policy is the most intractable problem we have been faced with, but the most difficult and important is this analysis of the government's strategy". Perhaps today he would reassess the relative intractability of the subjects under study.

In general the subjects which the Cabinet has referred to the CPRS are confidential—as are the recommendations made. But it is known that apart from government research funding, inflation and energy it has studied Concorde, the British computer industry, the Post Office giro, whether there is an optimum population level for the nation, multinational companies and the bane of all governments, regional policy.

The think-tank has, under various circumstances, published three of its 'thinks'. There was (and is) the redistribution of government research funds which threw Lord Rothschild himself into public prominence and is still somewhat controversial. He has continued to maintain that before the White Paper's adoption "the organisation of government science was wrong" but has subsequently admitted "the only real regret I have is that I did not explain the customer/contractor principle. I didn't explain it because I thought it self evident. But it evidently was not so." If he had, this would have made the report three times as long but perhaps "some of the scientists would have been less hostile".

The study on population has also been published and most recently the think-tank's long gestated views on energy were drilled out of the government machine by the Secretary of State for Energy, Mr Eric Varley. Nonetheless the think-tank published first and its emphatic reliance on saving energy rather than flirting with alternative energy sources was firmly lodged in the Department of Energy's paper which appeared a fortnight later. Particularly appealing features of the CPRS's *Energy Conservation* were the emphasis on an industrialists' 'annual energy audit' the possibility of saving substantially on electric light bulbs and the criticisms of large, fast uneconomic private cars. It would be a fitting memorial to Lord Rothschild's quirky reign in Whitehall if he could get public acceptance of a slow, small economical battery-driven urban runabout.

In the meantime, it seems that the CPRS is being downgraded with Rothschild's departure. Mr Wilson has already appointed a parallel unit of about eight advisers, currently untitled, but effectively an economics think-tank within No. 10 Downing Street itself headed by Dr Bernard Donoghue.

Those babies still pose problems

by Miranda Robertson

IT has not been entirely clear from press reports over the past two weeks that no child in the immediately foreseeable future is likely to have been in any sense a test tube baby. The most that is contemplated is the occasional emergence—in the traditional manner, from a woman—of a baby which spent its first few days as a fertilised ovum in a Petri dish (test tubes are not used for this kind of thing). That much is in principle quite possible and even desirable, although there are good reasons for supposing, in spite of claims at the recent British Medical Association meeting in Hull, that it may not yet have happened.

Scepticism about Professor Bevis's announcement could only be abolished by the publication of a technique which proved more successful than those already reported. The existence of three children, even if they were born to couples who had taken part in artificial inovulation trials, cannot vindicate the claim: where the wife is the egg donor and the husband the sperm donor, there is no way of telling whether the baby developed from the reimplanted egg, or whether it was naturally conceived in the course of the experiment, as can sometimes happen with presumed infertile couples.

The transfer of eggs fertilised outside the body into the uterus to complete development is already routine in laboratory animals used in genetic and developmental research. It is somewhat trickier to get eggs from humans than it is from mice or rabbits, but even so the techniques for obtaining viable fertilised human ova, developed over the past few years in Britain by Mr Patrick Steptoe of the Oldham and District General Hospital, and Dr Robert G. Edwards, of the University of Cambridge, are now extremely reliable. The problems which have prevented the animal success from being extended to humans lie not within the embryo but with the reproductive physiology of the mother in whose uterus the embryo must be able to implant.

It is not particularly surprising that the fourteen egg transfers so far reported by Mr Steptoe have failed. Even in mice, the failure rate for implantation of transferred eggs is something like 50%. There is every reason to suppose that some proportion of all fertilised eggs in normal women fail to implant and are flushed out, unnoticed, with the next menstual flow. Patients whose infertility is an indication for artificial inovulation are not normal women. The blocked oviducts which makes the procedure necessary are likely to be the consequence of disease which may have had other effects on the reproductive apparatus. The very procedure of injecting the fertilised ovum into the womb may initiate contractions which militate against implantation.

Finally, the hormonal interaction between the mother and the embryo is both critical and ill understood. In order to control the timing of reimplantation of the ovum, the woman's cycle is induced artificially with hormones, and this may result in endocrine deficiencies which could account for the failures. Nor is it clear what is the optional point at which reimplantation should be attempted.

Clearly, it is only a matter of time before someone produces a baby which began its life in laboratory glassware. That is a realistic and human goal according to Dr Edwards: "To give a couple their own child obviously needs no justification" and fears about the possible biological hazards and social implications of the technique seem on the whole unwarranted.

The misgivings voiced by the Medical Research Council on the abnormalities in embryos fertilised *in vitro* do not seem to be substantiated by the considerable body of data on animals (*Nature*, **244**, 333; 1973). The essential point is that the embryo before implantation is undifferentiated and thus almost impervious to injury. If enough cells are killed during the manipulations, the embryo dies. If it does not die, the cells regulate to produce a normal organism. There is a slight danger of triploidy if the ovum is fertilised by more than one sperm, but this can be avoided by proper control of the number of sperm used to fertilise one egg.

Most of the social, legal and ethical issues raised in connection with fertilisation *in vitro* differ little from those which arise in connection with artificial insemination or with adoption. The more horrendous imaginings of determined doomwatchers can probably be dismissed under the general proposition that it does not follow that once a thing is feasible it will necessarily or even probably be turned to undesirable ends.

These arguments, both on the feasibility and on the desirability of the technology for growing embryos *in vitro*, apply only to preimplantation development. The human uterus is still irreplaceable between six days and 24 weeks of gestation, which includes the most critical developmental period—that of differentation, when the foetus is at its most susceptible to teratogenic influences. Not only are the problems inherent in the creation of an artificial womb during this period enormous, but none of the stated aims of current research on human embryos *in vitro* would be served by it. The immediate aims as seen by Mr Steptoe are to overcome infertility in the 2% of couples in which it is caused by blocked oviducts, to control sex-linked disease by selecting fertilised eggs of one sex only for reimplantation, to investigate the causes of abnormality in preimplantation embyros, and to test male contraceptive drugs which can only be evaluated on the basis of their ability to prevent sperm from fertilising eggs. None of these goals would be advanced by the development at vast expense of an elaborate surrogate uterus. Not only does Mr Steptoe see no medical justification for carrying development *in vitro* further than the first few days, but the danger of abnormalities in the foetus does become serious once differentiation begins. □

Crisis in science education

from Peter Lindon

AN unhappy conjunction of two events was the origin of a conference on The Crisis in Engineering and Science Education in the West held at The University of Manchester Institute of Science and Technology (UMIST) on July 16 and 17. The two events in question were the 150th anniversary of the Institute and the decline in applications for science and engineering in the universities.

A sense of gloom and impending disaster was created from the beginning as Lord Bowden the Principal of UMIST predicted bankruptcy within 18 months for universities in the absence of any change in government policies. With student applications having dramatically fallen, and classes containing an ever increasing proportion of foreign students he wondered where the engineers and scientists to drive our essential industries would be coming from. The mechanism of manpower supply and demand seemed to be seriously at fault. From the time a child made decisions affecting the future direction of his studies until the time when he qualified, some 8–10 years had elapsed: a lag which must be an inherent cause of instability in recruitment to industry and enrolment at universities. It is precisely at the time when the need for scientists is greatest that the numbers and quality of science school teachers are eroded, the children become disillusioned with their experience of science in the schools, and the manpower supply is shut off.

Some contributors did not consider this mechanistic explanation sufficient. The social influences upon student choice were also important and Sir

Sea water for irrigation

by our Soviet Correspondent

A DETAILED analysis of the surface waters of the Eastern Baltic and the Gulf of Finland has now provided a theoretical basis for an experiment begun with field trials in 1969—the use of these water masses as a source of irrigation.

The first trials took place on the island of Mukku (Estonian SSR). The irrigated land yielded the "hoped-for" results, and in the spring of 1970 the research programme was started by the Irrigation Section of the V.I. Lenin All-Union Academy of Agricultural Sciences. A systematic study was made of the salinity and mineral content of the coastal waters of northern and western Estonia and of the Khiiumaa and Sarremaa. In all, 63 coastal 'points' were tested, samples being taken from depths of 50 cm to 1 m. In all, 664 specimens of water were analysed.

According to the report published in *Pravda* (July 25, 1974), the average salinity for the area was 1.5–2.0 g l^{-1} ("almost fit for drinking"—a not surprising result, since the low salinity of the Baltic is a well known phenomenon, particularly as regards the 30–50 cm and 50–60 cm layers). Also in 1970, field tests were begun on three collective farms having contrasting soils (sandy, arigillaceous and soddy-calcareous), using crops of perennial grass, barley, potatoes, swedes, early and late cabbage, table beet, cucumbers and so on. During the four years of the tests, good results have been obtained with pasture grasses, barley and a number of vegetables. Subsequent soil tests indicated the introduction of "a fairly considerable quantity" of dissolved mineral salts into the soil; in particular, after one summer of irrigation with sea water, the sodium content of the arable soil of the "Linnamyae" state farm (of soddy-calcareous type) rose from 4–10 mg per 100 g soil to 30 mg per 100 g soil. In 1971–73 further tests were carried out to determine the effect of the increased salinity on the soil microflora.

According to the *Pravda* report, which is signed by no less a person than O. Valing, Chairman of the State Committee of the Council of Ministers of the Estonian SSR on Land Reclamation and Water Economy, the results indicate that, "with a proper choice of crops and agricultural technology", the use of sea water in irrigation can prove an important adjunct to Estonian agriculture. Yet some facts, which he calls "curious" seem to indicate the need for caution in assessing the nutritional aspects of these results. Thus barley grows taller and gives a heavier grain yield, when grown on sea water, but the protein content is "somewhat" lower. In the case of cabbage and beet, the sugar content of the sea water crop is higher. And the presentation of the report in *Pravda*, on page 2, which is normally reserved for "party" news (science reports normally are found on page 3 or page 6) would suggest that the original decision to use sea water might well have been of political, rather than scientific, origin. When one recalls the disastrous effect on Soviet agriculture, and Soviet science as a whole, of another such political decision—Lysenkoism—one can only await future news from the fields of Estonia with a certain caution.

Frederick Dainton, chairman of the University Grants Commission, referred to the alienation from science caused by its unacceptable relationship with power (often misused), and its lack of concern for people. The exploitation of science in the military context and the connection between industrialisation and pollution were cited as examples. Sir Frederick believed that science should be taught in the universities as a humanity: not as an instrument of control over the environment, but as a subject of interest for enquiring minds.

It was reassuring to find that Britain is not alone in experiencing difficulties, the most frequently recurring theme being the effects of inflation which militate so strongly against educational interests. Relief from the prevailing atmosphere of doom was given by Dr W. B. Lewis in describing changes in science at Canadian universities. Here, one felt, was a country with security in its possession of large natural resources and confidence in its future. Despite earlier predictions that the need for scientists would decline, the numbers of graduates and PhDs had increased enormously in 1973 and all were now in relevant employment. Asked what had made such changes possible, Dr Lewis stressed the prime importance of cheap energy which in Canada was not simply derived from hydroelectric sources. The advanced energy programme was capable of producing electricity even more cheaply than from water power.

Dr J. B. Adams of CRN believed the qualities required of graduates were job flexibility and the abilities to plan, budget and acquire new technologies. He praised the breadth of approach found in general applied physics degrees and made the interesting observation that 40% of the engineers in his organisation were in fact physics graduates. The point was taken up by Professor H. B. G, Casimir, the President of the Royal Netherlands Academy of Sciences and Letters who believed that the only purpose of specialisation was to inculate the ability to specialise; the specific subject was only of secondary importance. The three year degree in England was too short to make specialisation within it a sound proposition and he suggested that industry in this country should be prepared to recruit far more PhDs.

Sir Frederick Dainton also had some harsh things to say about specialisation in the first degree. A curriculum based on professional requirements was the easy way out for university teachers who were subject specialists themselves. Yet the factual and professional content of a degree was ephemeral. What was needed was the ability to think logically and transmit ideas clearly. Science should be taught in a spirit of enquiry and more time should be devoted by faculty to reflective consideration of their subjects and the development of discovery-based learning techniques, instead of trivial research.

Crisis is Greek for decision. Predictably the conference did not produce one, though it did illuminate some of the underlying problems of university science. With issues of such complexity and gravity one wished the organisers had provided more opportunity for discussion in smaller groups. Half-hour discussions with 450 people all facing in the same direction rarely produce anything of substance. And what of the school-teachers, invited in large numbers yet unrepresented by speakers? They work amongst shifting sands; the science departments get poorer recruitment every year and the competition from what they see as undemanding soft options gets fiercer. At a time when even previously highly selective university departments are touting for undergraduates in the schools they needed some guidance on whether to try and stimulate schoolchildren to take up science or steer them away from it. None of the speakers, one felt, took this section of the audience very seriously.

What did emerge was the fundamental need to recover the ability to plan in the long term. Only in this way can there be orderly development and the maintaining of morale. Inflation has negated planning, and if it cannot be cured we must at least learn to live with it more successfully. Meanwhile we seem confined to the world of Kafka's aphorism: "There is an end but no way; what we call the way is shilly-shallying". □

Patrick Blackett . . .

an appreciation by Sir Edward Bullard

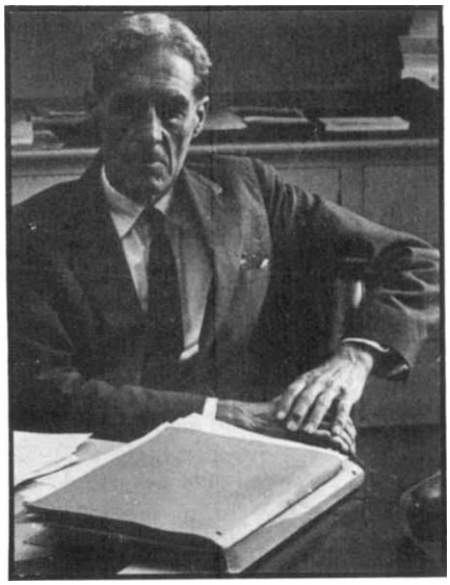
PATRICK BLACKETT, who died on July 13, was I suppose, the most versatile and the best loved physicist of his generation. His achievement was also without rival. Few of his friends had seen much of him during the past two years, but the sense of loss is, for me at any rate, unique. When J. J. Thomson and Rutherford died, it was sad and it was the end of an era, but their work was done and they had not, in the same degree, the wide ranging interest or the personal concern or kindness that were so characteristic of Blackett and which lasted unabated into old age.

Blackett was born in November 1897, the son of a stockbroker. At an early age he went to the Royal Naval College at Osborne and then to Dartmouth and to sea at the beginning of the 1914–18 war.

After the war he was sent to Cambridge by the Navy and soon decided that he did not want to return to the service. He once told me how, after this difficult decision, he felt as if a great weight had been lifted. He had all his life, a great affection for the Navy and a liking for an active life, but he felt he needed a wider field and one where the conventions as to what it was suitable to say and do were less restrictive.

I do not know exactly how he transformed himself from a naval officer to a research worker in the Cavendish, there is no mention in *Who's Who* of his taking a BA. He never attempted to take a PhD and when teased about accepting a peerage, replied that at any rate he had remained Mr Blackett until after he retired (this was not strictly true as he had a large number of honorary doctorates). He soon obtained striking results in the Cavendish planning of the postwar development of an automatic cloud chamber. C. T. R. Wilson, who first devised the cloud chamber before the war, was a slow-working perfectionist; Blackett was willing to take tens of thousands of photographs of hundreds of thousands of tracks if the problem needed that many. In 1925 he succeeded in photographing the emission of a proton from ^{14}N in a collision with an α particle. These famous photographs have adorned almost every text book of nuclear physics for the past fifty years.

The work with the cloud chamber led to the discovery of the positive electron in 1933 and to the study of cosmic rays by a counter-controlled chamber. This work brought out Blackett's remarkable facility in instrument



design. He could use physical theory to design a piece of equipment and then draw it, make it and get it to work. I have known nobody who possessed this combination of talents in the degree that he did. It is a major theme in his scientific work and can be seen in the bomb-sight he designed at Farnborough and in the magnetometer which he and his students used so effectively in the 1950s.

He was not entirely at ease in Cambridge or in the Cavendish. He felt that Rutherford gave a disproportionate share of support and funds to Kapitza and, perhaps, that his own left wing politics did not fit well in the still feudal-Victorian environment of Cambridge. He left for Birkbeck College in 1933, where he continued his work on cosmic rays, and then moved to Manchester in 1937. At this time he became involved in the controversies concerning the air defence of Great Britain and the relative importance of radar and other methods of detection. As a member of Tizard's Air Defence Committee he played an important part in ensuring that the essential stations were working when they were needed in 1940.

Soon after the outbreak of war he was at Farnborough; from there he moved to Coastal Command. There, and later at the Admiralty, he and E. J. Williams essentially invented operational research. He thought of it as the study of what actually happens as opposed to what is supposed or desired to happen. It was work that suited him; it involved not only intelligence and ingenuity but firmness, persuasiveness and a commanding personality. The effect on the U-boat war was of great importance.

In the course of his work at the Admiralty he became convinced of the ineffectiveness of the area bombing of cities. After the war this argument became connected with questions of nuclear strategy. He, perhaps unwisely, said that the idea of winning a war by the wholesale destruction of cities and the slaughter of civilians was an Anglo-American innovation and was not shared by the Germans or the Russians. This argument culminated in his arrest by a sheriff in some city in the Southern United States where his plane had made a refuelling stop on the way from Mexico to Canada (the sheriff was very civil and took him to his own house and not to jail).

In the last four months of the war Blackett became deeply involved in the planning of the postwar development of science, the rapidity of the subsequent development is in large measure due to him. He returned to Manchester in 1945 and started on a completely new line of work. He investigated a possible relation between rotation and magnetisation and, when this failed as a theory of the Earth's magnetic field, turned to the study of rock magnetism. The part that this work played in the modern developments of geological theory cannot be described here.

In the Attlee administration he was regarded as too far to the left to be an acceptable advisor or member of the government. In Mr Wilson's first administration he played a large part in the setting up and conduct of the Ministry of Technology. He was a member of many official and unofficial committees, his work as Chairman of the Council of the Department of Scientific and Industrial Research (DSIR) in 1955–60 was of particular importance for the support of science in the universities.

The last time I saw Blackett I remarked that his portrait at the Royal Society made him look very serious. He replied: "But I am a very serious man". He was; he minded about the things he considered important, he thought deeply about them and held his views firmly. This could have made him tiresome and a bore, but it didn't: he was wonderfully intelligent, charming, fun to be with, dignified and handsome. There was no one like him. He had every kind of honour; the Presidency of the Royal Society, a Nobel Prize, a peerage, all the medals and honorary doctorates a man could ask for, membership of the American, French and Russian Academies and many more. To add to all this he was married to one of the most delightful women in the world who did much to prevent him from becoming too serious.

correspondence

Academics in Chile

SIR,—As signatories with other colleagues of a letter on academic life in Chile (*Nature*, May 29) we feel it necessary to comment on Dr Eyzaguirre's answer which appeared in the issue of July 5.

Dr Eyzaguirre says our letter was inaccurate, although he concedes that academic people were killed or persecuted because of "actively" opposing the military junta or because of "denunciation by neighbours or colleagues". We would like to point out the omissions and inaccuracies we find in Dr Eyzaguirre's letter.

(1) Dr Eyzaguirre depicts Dr Allende, the legally elected president of Chile, as a "corrupt" politician whose government was responsible for "chaos and anarchy". He omits to say however, that Dr Allende's government strictly respected freedom of speech and of the press and that nobody was killed, imprisoned or persecuted because of his ideas. Another omission concerns Dr Allende's respect for the independence of the university, exemplified by the election as Rector of the University of Chile of Professor Boeninger, well known to be an opponent of the government.

(2) Dr Eyzaguirre's eulogy of the military coup ("the military struck with vigour") is shocking. He admits that some "unpleasant" excesses were committed. But nothing is said about the killings in the working class quarters (*poblaciones*), the hunting of the foreigners, the tortures and the shootings at the stadiums, and the 'purification' of the libraries followed by bonfires of 'forbidden' books. The authenticity of these events is not in doubt since they have been repeatedly denounced by non-Marxist organisations including Amnesty International, the World Council of Churches and the Conference of Chilean Catholic Bishops.

(3) The comparison that Dr Eyzaguirre draws between the nomination of military chiefs as university rectors in Chile, with the nomination, for example, of General Eisenhower as President of Columbia University would be funny if it were not so sad. In the second case, the university chose to honour an outstanding military and political personality, whereas in the first a military group was imposed on the university to ensure the rigid political control of the faculty and the students.

(4) Dr Eyzaguirre states that "wholesale executions of faculty and scientists have not occurred" and that "very few fell either immediately after the coup because, allegedly, were caught either shooting at soldiers, carrying arms or actively participating in the organisation of revolutionary activities". Some examples may illustrate the inaccuracy of such statement. Professors Ramirez and Peña, after being sentenced by the military to only two months in prison, were shot in the city of La Serena. Leopoldo Benitez, an architect, of the Catholic University and Victor Jara, a musician, of the Technological University were assassinated in cold blood at the Stadium Chile. Enrique Paris and Luis Sanguinetti of the University of Chile at Santiago and Michael Woodward, of Valparaiso, died while being tortured. More than ten medical doctors were shot by the junta.

(5) Dr Eyzaguirre takes as an example of the university situation the Catholic University, where no student has been expelled. He says that Admiral Swett Madge, the new Rector, was nominated with the approval of Cardinal Silva Enriquez. He omits to say that the previous Rector, Professor Fernando Castillo Velazco, also approved by the Cardinal, was dismissed by the junta, had his house ransacked and decided to leave Chile. Dr Eyzaguirre also states that only 3.5% of the Faculty was dismissed by the Rector on political grounds. He omits to say that at least four institutes (among them the Institute of Economic History and the CEREN, a centre for Chilean studies) were dismantled and all their members dismissed. He also neglects to say that Father Mauricio Hebert and six other professors were expelled from the country.

Moreover the Catholic University enrols only 10% of the student population. Dr Eyzaguirre does not quote the number of faculty members dismissed at the University of Chile. He only tells us that the only students expelled (no figure is given here either) were those with unsatisfactory academic records. It is difficult to accept, for instance, that more than half (141 out of 254) of the first-year students in the science school, who were not allowed to re-register, were agitators or professional politicians. A final remark is necessary. When we signed the letter to *Nature* asking for help for the jobless university teachers, and teachers who were persecuted by the junta or who refused to work under a totalitarian regime, we did not state or imply that the scientists or academics who decided to stay in Chile (among them many respected friends) were fascists. Dr Eyzaguirre says that they are "labouring under difficult circumstances which are mainly economic". We think that this is an understatement in view of the present lack of freedom and disrespect of the human rights. It is because of this that we find it so unfair that Dr Eyzaguirre should dub those who left Chile as '*gauchistes de salon*', in particular because many of them still have families in Chile and are therefore unable to answer him publicly.

Yours faithfully,

H. M. GERSCHENFELD
J. P. CHANGEUX

Paris, France

Algae as fuel

SIR,—A few words may be in order about planktonic algae cultivation as a means of harnessing solar energy, which was not mentioned in your Energy Review (*Nature*, June 21, 1974).

Algal cultivation has many advantages over plant cultivation. Whereas land for afforestation is becoming scarce, vast areas of lagoons and inland lakes are available for algal cultivation. About 1% of sunlight that falls on an area is absorbed by the plants growing there, but sunlight can diffuse through water down to a few hundred feet and so more than 1% of the sunlight can ultimately be absorbed by algae in water. Algae can grow in a variety of aquatic environments and there will be added advantages if sewage is added to the medium because the oxygen liberated by algal photosynthesis can be used by the bacteria in the medium to break down the organic matter in the sewage.

Also algae multiply fast. In large experimental ponds yields as high as 36 tons of algae per acre of pond per year have been achieved, compared with the yield of about 1 ton per acre of dry organic matter which plants yield. Thus a lake of 100 square miles can yield more than 2,000,000 tons of algae per year, which can be used as fuel in conventional thermal power stations.

Yours faithfully,

N. UMAKANTHA

Karnatak University,
Dharwar, India

news and views

Symmetry in recognition sites for proteins

WHEN nucleotide sequence analysis on RNA was beginning to gather momentum in 1965, such endeavours with even the simplest of DNA molecules were limited to preliminary skirmishes from a few optimists; but it was at this time that Bernardi entertained a model for the interaction of spleen DNase with its substrate which is now widely invoked for the reaction of many proteins with DNA. From the nature of the 3′ termini of the products generated by this enzyme, which contained two identical polypeptide chains, Bernardi (*Adv. Enzym.*, **31**, 13; 1968) proposed that the subunits of the enzyme are associated about an axis of two-fold rotational symmetry. Thus alignment of this axis with a corresponding axis between two opposed –G–C– pairs in a DNA duplex ensured that a catalytic site on each enzyme subunit is appropriately positioned to make equivalent breaks in each polynucleotide chain to leave –Gp.

Interactions based on this type of model proved very attractive for the more complex nucleotide sequences found at the positions of breakage of DNA by certain restriction enzymes. Following the demonstration by Kelly and Smith (*J. molec. biol.*, **51**, 393; 1970) that nucleotide sequences at the sites of breakage of DNA by a restriction enzyme (comprising four subunits) from *Haemophilus influenzae* were bisected by an axis of two-fold rotational symmetry, such rotationally symmetrical sequences, or palindromes as they are termed in some quarters, have been found at the interaction sites for several restriction and modification enzymes: the recognition sequences so far determined are tetra, penta, or hexanucleotides, becoming degenerate thereafter.

One would anticipate that some of the more important control functions would require larger nucleotide sequences to ensure their unique recognition. It was therefore of interest to note that in the first of the larger recognition sequences to be determined, that of the interaction site for the λ *ter* system, that is, the enzyme generating mature DNA molecules from a concatenated replicative intermediate form (Weigel, Englund, Murray and Old, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1151; 1973), rotational symmetry was again apparent, but this time the base pairs that were symmetrically arranged about the axis were not in continuous sequence, but showed a broken, or hyphenated symmetry. The *lac* operator provided a further, and rather more complex, example of a sequence of nucleotides with a discontinuous symmetry about an axis through a base pair rather than between two pairs, a feature of one of the recognition sites of the restriction enzyme (Gilbert and Maxam, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3587; 1973).

Recent and current work on the structure of DNA in areas concerned with the control of gene expression is providing more examples of structures of this sort, and a fascinating picture is emerging of the rather complex way in which the macromolecules may interact. Detailed study of regulation in phage λ has been made possible by exploiting the extensive background of genetics with this phage and the availability of many deletion and mutant strains. Expression of early genes in λ begins with transcription from two promoters, P_L and P_R, located astride the immunity region. Interaction of RNA polymerase with these promoters is prevented by interaction of the λ repressor, the C_I gene product, with the relevant operators, O_L and O_R. Maniatis and Ptashne (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1531; 1973) recently showed this to be a rather complex reaction involving at each promoter the stepwise addition of repressor molecules to six reiterated binding sites which were similar but not identical in nucleotide sequence. These sites, S_1 to S_6 at the two operators, ran in opposite directions towards the C_I gene on the map of the λ chromosome. Other promoters mediate the expression of genes involved in late functions and yet another promoter dictates synthesis of repressor during the consolidation of lysogeny. The various promoters are accurately located on the map of the λ chromosome. This enabled Allet and Solem (*J. molec. Biol.*, **85**, 475; 1974), through a comparative analysis (by gel electrophoresis) of the fragments obtained by digestion of DNA from λ and a variety of deletion strains with several restriction endonucleases, to isolate fragments of DNA containing only one promoter; this work considerably enhances opportunities for biochemical studies of transcription *in vitro*.

Of more immediate interest, however, was their observation, made also by Maurer, Maniatis and Ptashne (*Nature*, **249**, 221; 1974), that DNA from the mutants *sex*1 and *sex*3 which are defective in the left promoters, P_L, when digested with restriction endonuclease R.HindII did not give the fragments that had been shown to result from breakage within O_L the left operator, but instead gave a larger fragment equivalent in size to the two missing fragments. A corresponding mutant, *x*13, inactivating the right promoter, P_R, did not, however, affect the action of R.HindII at its site located within O_R. Both groups of workers then showed that the sites for R.HindII located within both O_L and O_R were protected from digestion by the binding of RNA polymerase, which established that operator and promoter sequences are identical or overlapping.

The occurrence of the R.HindII recognition sequence, –G–T–Y–R–A–C–, within the operators O_L and O_R has now been exploited by Maniatis, Ptashne, Barrell and Donelson, (see page 394 of this issue of *Nature*) for determination of a significant piece of nucleotide sequence in this important area of the λ DNA molecule. The R.HindII fragments flanking the break point in O_L, which occurs between sites S_1 and S_2, are about 320 nucleotides long to the left and about 1,125 nucleotides long to the right. The longer of these two fragments was prepared in quantity (using gel electrophoresis), denatured, and then annealed to equimolar quantities of the purified *l*-strand of λ DNA. This gave a molecule that was both a primer and template for polymerase reactions, so that the 3′ terminus of the annealed strand of 1,125 fragment could be extended by condensation of nucleoside 5′-triphosphates with DNA polymerase. Using Berg's adaptation of this reaction to substitute incorporation of a ribonucleotide for one of the deoxyribonucleotides, Maniatis *et al.* made products with rC or rG substituted (in separate reactions) to facilitate analysis of ^{32}P-labelled extensions of the 1,125 fragment. Analysis of the products of these syntheses were simplified by first cleaving with the HindII restriction enzyme and fractionation of the mixture of oligonucleotides (which had

a common 5′ terminus but varied in their 3′ terminus because of asynchronous synthesis) by the homochromotographic methods exploited so well by Sanger and his colleagues. Further cleavage of these oligonucleotides with pancreatic or ribonuclease T_1, followed by fractionation of the products and analysis of them by partial exonuclease digestion (to give further products that could be identified from their behaviour on two-dimensional fractionation systems), finally led to the proposal of a sequence of 59 nucleotides from the HindII site in O_L which extends into the region of *N* gene transcription. Since this sequence originated at the HindII site within O_L which is protected by RNA polymerase binding, it limits the distance separating bound RNA polymerase from the T–A–G with which *N* gene transcription begins to 33 base pairs and so reinforces the challenge to the suggestion made two years ago that the left promoter and start of transcription are appreciably separated.

Within this region of 33 base pairs to the left of the HindII site are three axes of two-fold rotational symmetry each involving hyphenated distribution of the symmetrically arranged 14 to 16 bases. All three blocks of symmetrical sequence seem to lie within the region covered by the repressor and it is intriguing to contemplate that these interdigitating symmetries are the recognition sequences for different regulatory proteins (including the *tof* (or *cro*) gene product to regulate leftward transcription). One can only agree with the authors that analysis of the structure of the remaining sites in the operators and localisation of regulatory mutations should provide a clearer understanding of the significance of these symmetries.

Of no less interest will be the elucidation of the structure of other promoters (in addition to that of the phage fd promoters determined recently by Seeburg and Schaller), because Allet Roberts Gesteland and Solem (*Nature* **429,** 217; 1974) have shown that the HindII recognition sequence occurs in several, but not all, promoters in phages λ and T7 as well as in the mammalian viruses SV40 and adeno2. Extension of the work on the *lac* operator (Gilbert, Maizels and Maxam, *Cold Spring Harb. Symp. quant. Biol.*, **38,** 845; 1973) has now identified the position of several mutations in this sequence and these occur in both symmetrical and non-symmetrically arranged bases. With this system (and others) analyses of the regions of the enzyme involved in the interaction will also be relevant to the meaning of symmetry and here, production of active *lac* repressors that are covalently fused to β-galactosidase (Müller-Hill and Kania, *Nature*, **249,** 561; 1974) will help to define necessary regions of the repressor. Detailed analysis of the L-arabinose operon, which is under positive control, is also in prospect following the recent studies of araC protein binding to the *ara* operator by Wilcox, Clemetson, Cleary and Englesberg (*J. molec. Biol.*, **85,** 589; 1974). Much remains to interest those who do not believe that prokaryotic molecular biology is finished.

From a correspondent

Russian cosmology

It has been a recurring preoccupation among cosmologists to establish connections between the microscopic and macroscopic of the Universe. Eddington attempted to discover a fundamental relationship between atomic and cosmological systems, and other workers still continue to pursue this possibility. Among these workers is the nuclear physicist Andrei Sakharov, the same individual who has recently achieved world renown as a leading Soviet dissident. Sakharov and his colleagues, notably the eminent astro-

Sitting in a hot seat

The spectacular nature of volcanic eruptions has long attracted scientific observers. As far back as 79 AD Pliny documented the eruption of Vesuvius and the accompanying destruction of Pompeii and Herculaneum, and by the beginning of this century, when Frank Perret was travelling the world compiling a chronicle of volcanic eruptions, volcanology had become well established as a science.

Today, there are volcanic observatories in a large number of places throughout the world. Many, including those on Vesuvius, and in Japan, Hawaii and Soufriére in the West Indies, use extremely sophisticated techniques. Seismic networks record tell-tale cycles of seismic activity, and geodetic measurements involve the use of lasers to detect the dilations of a volcano, which presage serious eruptions.

Volcanologists are not confined to predicting the onset of eruptions. By taking records during eruptions they can determine the source and nature of a lava, which often enables them to anticipate how a particular volcano is likely to behave during future periods of activity. The prognosis may be of use to communities who wish to prepare as best they can for likely contingencies. In that respect, the measurement of such parameters as the viscosity, effusion rates and temperature of a lava, and the detailed chemical and mineralogical analyses are all important. On page 385 of this edition of *Nature* an Anglo-Italian team of volcanologists report their observations of a recent eruption of Mount Etna in Sicily.

Most volcanologists are quick to admit that, unfortunately, there is very little that can be done to safeguard livelihoods and property. On Hawaii, always threatened by mobile basaltic magmas, there have been attempts to chill advancing lavas, but these measures usually prove to be ineffective. The Hawaiians have, however, successfully diverted lava from its course with bombs; but elsewhere, in densely populated areas, that action may be a palliative which merely shifts the problem from one community to another.

There are few precautions that can be taken against the threat of ash falls, as evidenced on Heimaey in Iceland in 1973. Then, during the eruption of Eldfell, homes and land were completely blanketed by vast volumes of ash within a few days. And against the worst type of volcanic hazard—*nuées ardentes*—there is absolutely nothing that can be done. *Nuées ardentes* are hot avalanches of loose, explosive lava lubricated by constantly expanding gases and vapours. Because of their internal energy, they sweep down the flanks of volcanoes with tremendous speed, often flowing uphill and across barriers, burning all in their paths. In 1902 a *nuée ardente* swept down Mont Pele, on Martinique, and, within the space of a few minutes, annihilated the 30,000 inhabitants of the port of St Pierre.

Inevitably, volcanoes still retain many unknown characteristics, and continue to behave unpredictably. Volcanologists on Vesuvius are becoming "very worried". That volcano has a long history of particularly unpleasant eruptions, and all of the indications are that it should have erupted at least twice during the past 30 years or so. Geodetic measurements show that the flanks of Vesuvius are bulging to an alarming degree. Evidently, the Vesuvian volcanologists could do worse than consider their own counsel. They might also be wise to bear in mind recent events on Etna—in 1971 the volcanic observatory on that mountain was among the first obstacles to be swept away before the advancing lava.

Allan Piper

physicist Zeldovich, have been developing a theory which attempts to link the phenomenon of gravitation, which governs the motion of large scale systems, with the quantum structure of the vacuum (*Dokl. Akad. Nauk S.S.S.R.*, **771**, 70; 1967).

The modern quantum field theorist has a conception of a vacuum which differs greatly from that implied by the popular usage of the word. The absence of particles does not mean just 'empty space'. Rather one must imagine a continual interchange of energy between different forms, as 'virtual' particles of all types appear and disappear in transient fluctuations of incredibly short duration. The origin of this vacuum energy is analogous to the energy which prevents the electrons in an atom from collapsing into the nucleus.

Also in gravitation theory, empty space is rich with structure. The presence of a gravitational field is interpreted in the general theory of relativity as a curvature of space, that is, a distortion of the geometry. The stronger the gravitational field, the more the space geometry becomes distorted. The precise amount of distortion produced by a given gravitating mass (for example the Sun) is determined by the value of Newton's gravitational constant G. Although this value may be measured, it has long been a dream among physicists that Newton's constant might be derived in some way, or connected in a relationship with other, perhaps microscopic, constants of nature.

Sakharov has proposed a derivation of G based on an analogy between the vacuum and an elastic medium. In the same way that distorting the shape of a piece of rubber leads to a change in its internal energy so, Sakharov argues, a distortion of empty space by a gravitating mass leads to a change in the energy of the vacuum, as interpreted by quantum field theory. G may then be calculated as an 'elastic constant of space' from the theory of elementary particles. The expression for G given by Sakharov contains an unknown mass which corresponds to the heaviest allowed element particle. To get the right value for G, this mass must be about 10^{-5} g, or 10^{19} times as heavy as the proton!

Whatever the merits of this particular piece of work, the idea of appealing to elementary particle physics and quantum field theory in investgations of large gravitating systems is a fascinating one which is occupying the attention of other Soviet scientists at this time. Zeldovich had previously explored a possible connection between the quantum fluctations of the vacuum and the existence of a cosmological constant in Einstein's field equations of general relativity. A recent paper (*Phys. Lett.*, **50B**, 340–342; 1974) by Kobsarev, Okun and Zeldovich presents new work in the same spirit. This time, these authors turn their attention to the subject of symmetry breaking in elementary particle physics. It is well known that certain elementary particle processes violate the so-called CP symmetry; that is, symmetry under charge reversal and space (mirror) reflection. The Russians have applied a simple model due to T. D. Lee for CP violation to the subject of cosmology. The essence of their article is that, because of CP violation the vacuum acquires a domain structure loosely similar to that of a ferromagnet, adjacent domains differing by the sign of the CP violation. These domains form during the early stages (at about 10^{-5} s) of the hot big-bang at the beginning of the Universe, and arise (at random) because of the existence of causally separated regions which always occur in this type of model universe. Roughly speaking, each domain wants to form with the same sign of CP violation throughout, but it cannot know the sign in adjacent domains. As the universe expands, so do the domains.

The behaviour of a universe filled with such domains is drastically different from the conventional models. The authors consider the dynamics of the system to be dominated by the behaviour of the domain walls, which are in a state of tension, and have the peculiar equation $p=-2/3\ \varepsilon$ connecting the pressure p and the energy density ε. This relation leads to a model universe which expands like t^2, that is, accelerates to unlimited expansion, even in the case of the cosmological constant being zero, in contrast to the conventional models of this class which always decelerate as they expand.

As the authors point out, such a rapid expansion is not really consistent with present density observations of the Universe. More seriously, the gravitational field of the domain walls would be likely to destroy the observed isotropy of the cosmic microwave background radiation. For this reason, it is suggested that a mechanism must exist for removing the domain walls after about 10^{14} s. One possible such mechanism would be to connect the sign of CP violation with the expansion of the Universe, perhaps along the lines of the work by Ne'eman (*Int. J. theor. Phys.*, **3**, 1; 1970).

It is hard to know how seriously cosmological models of this type should be taken. As a general principle, however, it is important to appreciate that as accelerating machines become ever more expensive to build, the testing of theories in elementary particle physics by constructing cosmological models is becoming progressively more attractive.

P. C. W. Davies

Floating on high

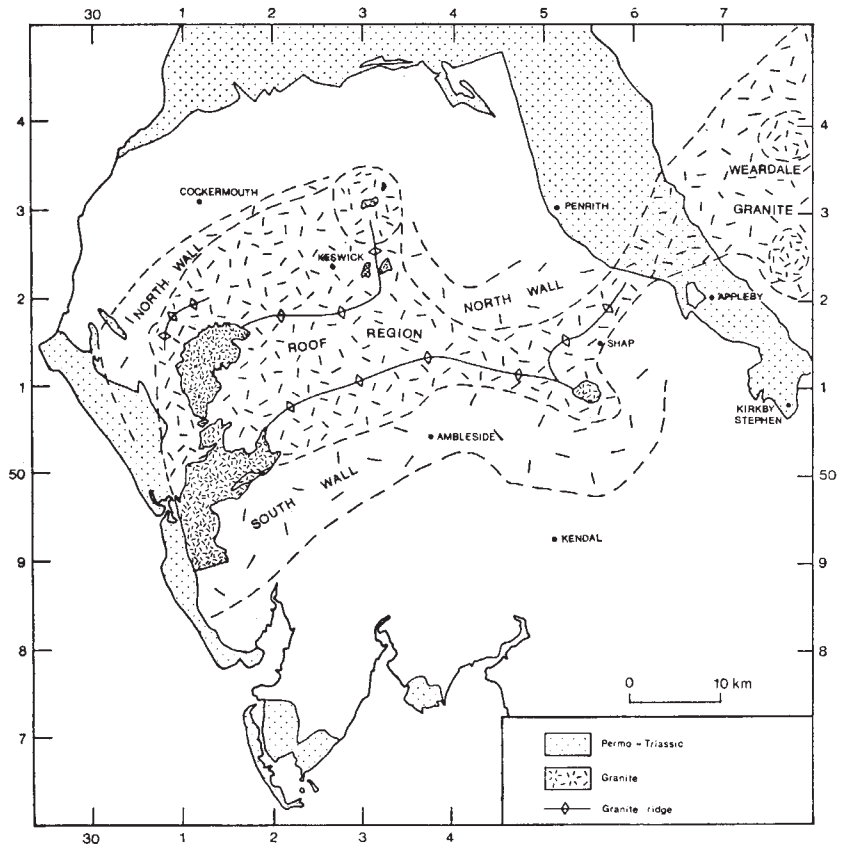
from J. Sutton

Seen from the air—a journey between Glasgow and London on a fine day provides the opportunity—the mountainous Lake District contrasts strikingly with the surrounding subdued landscape. This contrast has its roots in a long geological history which Bott has recently investigated (*J. geol. Soc. Lond.*, **130**, 309–331; 1974).

The Lake District, like many other areas of high ground in Britain, is underlain by Palaeozoic metamorphic rocks; the surrounding lower ground is underlain by younger rocks, though these too rest on a buried Palaeozoic basement. This situation, a deep Palaeozoic basement below a younger cover, can be matched elsewhere in and around Britain—below the Cheshire Plain for example.

During the past 250 million years, much of the crust of the British Isles has been broken into a mosaic of blocks some tens of kilometres across. Some of these have moved upwards, and others have gone down. Why should this be? That is a question which Bott has made his speciality in recent years. He has already shown that south-west England and the northern Pennines are underlain by granites which are larger than their surface exposures alone suggest. He has now demonstrated that the Lake District is in the same condition. Bott has established that the Lake District is marked by a belt of relatively low Bouguer anomalies; in other words, some rock near the surface is unusually light compared with its neighbours. Particularly low gravity values occur over the isolated granites which reach the surface at Eskdale, Shap and Skiddaw. Bott attributes the anomaly to a concealed granite, connecting these outcrops at depth, and extending for some 80 km, at least as far east as Weardale. The granite lies high in the crust, it is suggested, within the top 10 km, and thus forms another example of a high level Caledonian intrusion—it was intruded in early Devonian times, nearly 400 Myr ago.

These results bear on two distinct, but related, geological questions. In the first place the work brings out the position of outline of another Caledonian granite, adding to the number known already in north-west Britain. As Shackleton pointed out in the discussion of the article, the nature of the upper surface of the Lakeland granite presents a problem. Why is it that the levels to which the granite rose were rather uniform over quite a wide area? As more geophysical evidence on the shape and depth of other buried Palaeozoic granites comes to hand this



Sketch map showing the inferred distribution of unexposed and exposed granite beneath north-west England. The map shows the roof and wall regions of the postulated Lake District batholith, the main granite ridges being indicated by the gravity anomalies and the postulated connection to the Weardale granite (from Bott, *J. geol. Soc. Lond.*, **130**, 319; 1974).

problem might become better understood.

Such information will help with the second question—does the distribution of low density granities, high in the crust, play a critical part in determining which blocks of Palaeozoic crust act as bouyant upward-moving blocks and which subside to be buried by sediments? So much of the wealth of this country in the form of coal or oil is present in sedimentary basins formed since Carboniferous times above a Palaeozoic floor, that an understanding of this question would have a practical is well as an academic value.

Bott shows that in the Lake District the density contrast between the granite and the country rocks would cause a gravity deficiency of magnitude 1×10^{18}g, and finds that this is of the same order as the total mass of the Lake District rocks above sea level, which he computes as 1.49×10^{18}g. These calculations support the hypothesis which Bott has already applied at Dartmoor and in the Northern Pennines, which proposes that low density granites high in the crust can account for blocks of elevated country. There is good stratigraphical evidence that the mountainous block seen in the Lake District today, has been rising and shedding detritus at least since the Carboniferous. In other words, what is now the Lake District has been bouyant since the time the granite was intruded. An interesting experiment now would be to attempt to find out whether the depressed portions of Palaeozoic crust which underlie sedimentary basins lack low density intrusions. Such a discovery would give further support to Bott's hypothesis.

Radar mapping of the Moon

from David W. Hughes

WHY bother to look at the Moon with radar? The answer is quite simple. Whereas Earth-based telescopes can, with the best conditions of optical viewing, give a lunar surface resolution of a few hundred metres, returned radar echoes can yield the distribution of objects with sizes around the radar wavelength—typically in the 4–150 cm region. This degree of fine resolution can also be obtained using orbital and lunar surface photographic techniques but only at considerable expense.

Now a resolution of 1 arc s can be obtained at optical wavelengths, even using small telescopes. Using simple radar with currently available aerial beamwidths, and straightforward attempts at angular resolution, a value of 4 arc min is obtained, which when compared with the 30 arc min diameter of the lunar disk shows that only very large scale features can be picked out.

The Delay-Doppler mapping technique gets over this problem. As the Moon is rigid, a known motion of the whole body will enable the motion of each surface feature to be calculated. This movement produces a Doppler shift in the echo, and by high resolution frequency analysis selection can be made of the component of the echo originating from a known strip on the lunar surface; this strip is parallel to the apparent axis of rotation. Range analysis enables a circular annulus, centred on the sub-radar point, to be selected. A combination of both techniques isolates two scattering areas, only 10^{-5}, or less, of the total visible lunar surface. The ambiguity produced by having two areas is overcome by making the separation between these areas greater than the angular half power beam width of the radar antenna.

The resolution problem can be thought of in another way. Consider the radar wavefront hitting the Earth The apparent rotation of the Moon sweeps this wavefront past the antenna and so sequential sampling of the received signal produces results equivalent to sampling simultaneously the original waveform over the region swept out by the antenna during the time interval of observation. Thus, large observing apertures can be synthesised, producing greatly improved angular resolution; in fact the degree of resolution is at present only limited by system stability and data processing constraints.

The observed distribution of echo power with delay and Doppler frequency can be thought of as having two components. The first is coherent and quasispecular, caused by reflections from the relatively smooth undulating lunar surface. This component is polarised just like the incident radar beam. The second component is incoherent, diffuse and largely unpolarised, and originates from wavelength sized structures, thought to be mainly rocks on or near the surface. Careful normalisation of the returned echo enables surface tilt to be eliminated as a source of scattering difference between different regions of the lunar surface. The remaining enhancement of radar echoes is then a measure of the rock population (the rocks detected would have sizes between one quarter wavelength and about 10 wavelengths; rocks also give signals provided they are not buried deeper than about 20 wavelengths).

The results of high resolution radar mapping, at 3.8 cm and 70 cm, of the lunar surface facing the Earth are given in a series of three articles in a recent edition of *The Moon* (**10**, 1974). The

radar window is limited by the absorption by water and oxygen molecules in the Earth's atmosphere; 70 cm is in the centre of this window, and 3.8 cm is close to the edge. The 3.8 cm observations had a resolution of 2 km, and the 70 cm observations had an aerial resolution of 25 km^2, which is twenty times better than previous results. The results were obtained by Pettengill, Zisk and Catuna at the Haystack Observatory, Massachusetts Institute of Technology and by Thompson at the National Astronomy and Ionosphere Centre, Arecibo, Puerto Rico.

By comparing radar maps with Lunar Orbiter photographs of the same area three basic radar features can be distinguished. They are well defined, named craters, very small un-named craters and, finally, radar anomalies with no apparent optical counterpart. There seems to be an anomalously strong radar return from small craters, which is not necessarily correlated with their apparent size or optical albedo.

The authors conclude that the craters which give large echoes are young, with sharp contours and steep inner slopes. The rough inner surfaces are tilted steeply towards the Earth-based radar, leading to depolarisation of the returned signal. These craters also have excess rock populations caused by the excavation of large quantities of bedrock during formation and subsequent depositions around the crater in a strew field many kilometres across. Meteoric erosion slowly removes the hallow, grinds the rock into fine grain debris and produces a shallow subdued topography, thus reducing the echo strength to the average lunar value. Most of the enhanced echo craters are thought to be Copernican in age. It is concluded that spallation of rocks caused by impacts with sufficient energy to fracture centimetre sized and larger rocks, is a common lunar phenomenon.

One radar anomaly found in the Kepler region is thought to be formed by a large clod of material which was thrown out with the ray material when Kepler was formed. The 70 cm data, with the help of 'ground truth' observations at Apollo landing sites, has enabled the percentage roughness of the lunar surface to be calculated.

Not only are the youngest craters on the Moon strong radar scatterers; they also have enhanced eclipse temperatures (that is infrared anomalies—elevated temperatures which certain areas maintain during the umbral phase of an eclipse). Correlations between radar and infrared maps show that these 'hot spots' can be explained by the thermal behaviour of surface rocks, provided these are 10 cm or larger in size and are greater in number on the surface than at the Surveyor 1 and 3 landing sites.

Analysts celebrate

from T. S. West

THE celebrations of the centenary of the Society for Analytical Chemistry which were held from July 16 to 19, began with a special 'historical' meeting at the Royal Institution in London at which fraternal greetings from 30 kindred societies were presented—most of them from overseas. The scrolls and presentations are now on display for 6 months at the special exhibition "You and Your Analytical Chemist" at the Science Museum in South Kensington.

Sir Alan Hodgkin, president of the Royal Society, described the founding of the SAC on August 7, 1874 in the City Terminus Hotel, Cannon Street and traced the events that had been set in motion by the actions of Professor Theophilus Redwood and his group on that day. The society, which had formerly existed as The Society of Public Analysts (1874-1907), then as The Society of Public Analysts and Other Analytical Chemists (1907-1953), had a membership of 2,000 in 1972 when it had undertaken a joint role with the new amalgamated Chemical Society as the kernel of the Chemical Society's Analytical Division with a membership, in 1974, of 5,000.

Dr G. W. C. Milner, the centennial president of the SAC, highlighted some of the events of the past hundred years and looked forward to the future. The society, with only 2,000 members in its hundredth year obviously stood at the crossroads and was faced with a decision to continue, as before, independently or to amalgamate fully with three other British sister chemical societies (the former Chemical Society, The Faraday Society and the Royal Institute of Chemistry) and become the Analytical Division of the (new) Chemical Society. The presence of more than 3,000 additional 'first choice' members in the Analytical Division of the Chemical Society had influenced the council's decision to recommend full unification to the members at a referendum to be held later this year.

Nitrogen aggregates in diamond

from John Walker

IT has been known for many years that some diamonds contain precipitates ('platelets') on (100) crystal planes which give rise to anomalous features ('spikes') in Laue X-ray photographs. When Kaiser and Bond (*Phys. Rev.*, **115**, 857; 1959) found by mass spectrometry that these diamonds also contained large quantities of nitrogen impurity, it was natural to assume that it was contained in the platelets. Shortly afterwards, in 1962, the platelets were observed in transmission electron microscopy. Everything fitted and everybody was happy.

Six years later Sobolev *et al.* (*Sov. Phys. Doklady*, **12**, 665; 1968) upset the applecart by discovering that the X-ray spikes did not correlate with the total nitrogen content of diamonds, which meant that the platelets contained little if any nitrogen. This raises two questions. Of what are the platelets made and where is the nitrogen? The first question was and is still open, but the second could be partially answered. Electron microscopy can detect features only a few Ångstroms in diameter, and since none had been observed the nitrogen aggregates must be very small—effectively point defects. This deduction has now been confirmed by Turk and Klemens (*Phys. Rev. B*, **9**, 4422; 1974) using thermal conductivity.

Any imperfections in a crystal scatter the phonons (lattice vibrations) and decrease its thermal conductivity. The amount of scattering depends on how big the scattering centres are relative to the phonon wavelength, and since that changes with temperature, so does the scattering. Hence the variation of thermal conductivity with temperature contains information on the size and concentration of defects in a crystal. What Turk and Klemens have done is to calculate the scattering effect of platelets and point defects, and fit their results to the experimental thermal conductivity data of other workers. They deduce for one diamond that only 3% of the nitrogen it contains is in platelets: the rest is in small clusters of eight nitrogen atoms. They have therefore confirmed the conclusions of Sobolev *et al.* using a totally different technique, and the eight-atom cluster would probably be too small to see in the electron microscope, as was expected.

One would now hope that Turk and Klemens could extend their work to many more specimens: for example infrared and visible spectroscopy suggests that there are not one but at least three types of small nitrogen cluster (the so-called N3, A and B defects) in some diamonds. It would be interesting to see if they could be distinguished and their sizes measured.

Hierarchy of behaviours in a gastropod

from Marian Dawkins
Animal Behaviour Correspondent

ANIMALS in their natural environments are often exposed to mixed, even contradictory sensory inputs. It may be impossible for an animal to react to all these inputs at once, as the behaviours involved may actually be mutually incompatible; for example, an animal cannot both approach and withdraw from an object at the same time. One of the interesting features of the evolution of the behaviour of animals has therefore been the development of a system of priorities whereby these conflicts are resolved in such a way that the survival and reproduction of the animal is most likely. This often takes the form of animals in conflict situations performing certain behavioural responses to the partial or complete exclusion of others, and in this sense the animals can be said to have a hierarchy of behaviours.

Marine molluscs such as *Pleurobranchaea* have already proved to be extremely fruitful material for understanding the neurophysiological bases of many behaviours and it is therefore promising for the discovery of the neural basis of such a behavioural priority system that their behaviour has been found to be organised into an explicit priority sequence. Davis, Mpitsos and Pinneo (*J. comp. Physiol.*, **90**, 207–224; 225–243; 1974) have found that in this species, which is a carnivorous gastropod, feeding is a relatively dominant behaviour and takes precedence over most other behaviours. They presented animals with stimuli which released two different behaviours: in one experiment they gave a food stimulus to animals which had been turned upside down. Instead of showing their usual almost immediate righting behaviour, the animals stayed on their backs and ate, often for up to an hour. Feeding also dominated head withdrawal and mating, and Davis *et al.* argue that the adaptive significance of this is that *Pleurobranchaea* is a carnivore with a sporadic and rather limited food supply and so opportunities for feeding being rather scarce, feeding requires immediate and decisive interruption of all other behaviours.

Interestingly, there are some situations in which feeding is not given top priority. Davis *et al.* found that this behaviour is hormonally suppressed during egg laying; presumably this is an adaptation to ensure that the animals do not eat their own eggs. Even higher in the hierarchy than egg laying is the escape swimming response to avoid predators. This can interrupt any other behavioural act and so ensures that the animals survive to perform their other behaviours at times when no danger threatens.

The organisation of behaviours into hierarchies of adaptive priorities, Davis *et al.* suggest, may be a unifying theme for a diversity of complex behavioural phenomena in many animals, and to have discovered one in a mollusc with a simple analysable nervous system encourages the analysis of this phenomenon at the level of the single nerve cell.

Can partons be saved?

from David J. Miller

THE most eagerly anticipated parts of the International Conference on High Energy Physics, held in London during the first ten days of July, were the sessions devoted to electron–positron annihilation. Most delegates had already heard of the surprising results obtained by physicists at Stanford California, and at Cambridge Massachusets, concerning the rate of production of strongly interacting particles (hadrons). The conference gave the Stanford group a chance to report their results in detail, and to be closely cross-questioned about them. It also gave theorists their first chance to try and explain what had been discovered.

The Stanford and Cambridge experiments involved the storage of electron and positron beams of up to 2.6 GeV. The stored beams were brought together, twice per circuit in the Stanford SPEAR ring, and allowed to make head-on collisions. There was an array of scintillation counters and spark chambers around each intersection region, and it was possible to recognise whether outgoing charged tracks were caused by electrons, muons or hadrons. Electrons produce characteristic showers when they pass through dense material; hadrons are totally absorbed by a foot or so of steel, because of their strong interactions; muons are more penetrating than any other charged particles. The American results confirmed findings, at Frascati in Italy, Orsay in France and at Novosibirsk in Russia, that electron–positron scattering has all the properties predicted for it by the well established theory of quantum-electrodynamics. Elastic scattering, the production of muon pairs and the production of photon pairs all behaved exactly according to theory. The surprise came in the rate of hadron production. Up to a beam energy of about 1.5 GeV at Frascati, this rate falls off in the same way as the production of muon pairs. But in going from a total electron-positron energy of 3 GeV (that is 1.5 GeV electrons plus 1.5 GeV positrons, meeting head on) to the 5.2 GeV available at Stanford and Cambridge, the rate of hadron production levelled off and stayed roughly constant. This is not a contradiction of quantum electrodynamics, but it is very surprising.

Theorists had been quite happy with the behaviour of hadron production at the lower energies. They had compared it with muon production, and worked out a theory in which the electron and positron annihilated by first of all creating a massive single photon. This process is formally just the reverse of electron–positron pair production by a photon close to an atomic nucleus. The heavy photon could then generate a muon pair, one positive and one negative, or it might generate a 'parton' pair in just the same way. Partons are hypothetical objects which are supposed to be the building blocks from which the hadrons are made—just as the hadrons themselves are the building blocks for nuclei.

The parton theories have been supported by a number of recent experiments where leptons (electrons, muons or neutrinos) are scattered from nucleons (protons or neutrons, free or in nuclei). These so called deep inelastic scattering experiments have been done with electrons at a number of laboratories, including Stanford, and with neutrinos in the Gargamelle bubble chamber at CERN, Geneva. The results are in some ways similar to Geiger and Marsden's alpha particle scattering experiment, which led Rutherford in 1911 to postulate a small point nucleus in each atom. The angular distribution in deep inelastic lepton scattering from a proton is just what would be expected if protons had very small constituents. There is even evidence to suggest that these constituents or partons may have the same charge and spin as the quarks, which were postulated in 1973 by Gell-Mann and Zweig to explain the regularities in the spectrum of the resonances of the strongly interacting particles. The quarks are the most intuitively straightforward way of building up hadrons which fit into the 'SU(3)' symmetry scheme.

In 'parton pair production', by a single photon from electron–positron annihilation, it was suggested that the partons would first be produced, and would then 'clothe' themselves with other partons, spontaneously generated from the vacuum, forming hadrons of the kind which can be observed. Quarks, or any other form of parton, have never been observed, and perhaps cannot exist as free observable par-

ticles. Despite the complications of the 'clothing' process, the basic rate for hadron production by means of parton pair production should have the same dependence on the overall electron–positron energy as does muon pair production.

The new SPEAR and Cambridge results disagree with this. Muon production falls as the energy increases, but hadron production levels off. This directly contradicts simple parton theories. More than seventy theoretical papers were submitted to the conference, attempting to explain this effect in one way or another. Some suggest that the levelling off in hadron production is a temporary phenomenon at 'medium energies', and the high energy decrease has yet to begin. Others claim that massive 'charmed' particles are being produced (see *Nature*, **250**, 186; 1974 for a discussion of other contexts where 'charm' has been invoked), and the reaction rate is rising as more charmed final states become available. Other theorists have pointed out that the levelling off in hadron production is "more parton-like than partons". If the hadrons were not formed by production of pairs of point-like constituents, then the hadron production should fall faster than the rate of muon production. This suggests that some totally new phenomenon is being seen in electron–positron annihilation.

To test these theories it will be necessary to collect data at higher energies, and to make more detailed studies of the hadrons in the final state. The SPEAR ring is already being modified to give higher beam energies, but it seems likely that a much larger increase in energy will be needed to answer all the questions raised by the new data. British physicists could have a major part to play in the development of these studies. To reach the higher energies, new machines must be built, and Britain's long-term plans in this direction are as far advanced as those of any other nation. The Science Research Council should decide this year whether to build its EPIC storage rings which will have 14 GeV electrons colliding with 14 GeV positrons, a substantial step up from the energy of SPEAR. The Americans have similar plans but their new machine, called PEP, has also not been funded yet. If the United States does go ahead soon, PEP may be built a little more rapidly than EPIC since it can get its beams from the existing 20 GeV Stanford linear accelerator that now feeds SPEAR and other experimental facilities. But it takes a few years to do all the important experiments on a new accelerator, and there is plenty of room for two machines if they start work within a year or so of one another.

East African rift as membrane effect

from Peter J. Smith
Geomagnetism Correspondent

IT is almost exactly a year since Turcotte and Oxburgh (*Nature*, **244**, 337; 1973) first sketched out their ideas on the nature of mid-plate tectonics. For some time before this (and even since), numerous workers had sought to explain the origin of major tectonic activity away from plate margins in the relatively *ad hoc* terms of hot spots, with or without mantle plumes. The Hawaiian–Emperor island chain, for example, was seen as the surface trace resulting from the motion of the Pacific plate over a stationary or near-stationary magma source—a not unreasonable explanation, although one difficult to substantiate in the face of older and equally plausible interpretations based on less contentious terrestrial phenomena. On the other hand interpretations of continental mid-plate tectonic features in terms of hot spots were much less successful. How, for example, could hot spots be held to account for the extension across graben and rift valleys?

In their original summary article, Turcotte and Oxburgh were able to abolish many such problems at a stroke, ironically by returning to explanations involving more traditional concepts. They argued that major mid-plate tectonic activity results from crustal extension due to tensional stresses. Clearly this is likely to offer a more satisfactory explanation for the finite extension of rift valleys, for extension would be required to relieve the stresses. In the case of the Hawaiian islands, it meant returning to the much older, though far from disproved, idea that the chain is a "propagating tensional fracture in the lithosphere which causes volcanic activity as it propagates".

But Turcotte and Oxburgh not only were to propose the mechanism (crustal extension), they were also able to suggest two plausible ways in which the required tensional stresses could be produced. The first type of stress, the theory of which was developed by Turcotte (*Geophys. J.*, **36**, 33; 1974), is the membrane stress arising from non-spherical plate tectonics. Because the Earth is not quite spherical, a lithospheric plate must deform as its latitude changes, producing regions of both compression and tension involving stresses of up to at least several kilobars. Second, there are thermal stresses, now discussed in greater detail by Turcotte (*J. geophys. Res.*, **79**, 257; 1974). As oceanic lithosphere moves away from the ridges, non-uniform cooling will lead to thermal stresses unless there is relief by plastic flow. In fact, the upper lithosphere behaves elastically on geological time scales; Turcotte therefore concludes that thermal stresses up to 4 kbar (sufficiently large to fracture the lithosphere) may develop where the temperature is lower than about 300°C.

Presumably the forces giving rise to mid-plate tectonics generally arise from a complicated combination of membrane and thermal stresses, although the latter are clearly of particular relevance to the ocean floor. Indeed, in his most recent discussion in the *Journal of Geophysical Research*, Turcotte has suggested that even the transform faults and their associated fracture zones are contraction cracks which relieve the thermal stresses in the cooling lithosphere. In contrast to some other origins previously proposed for transform faults (for example, those involving small changes in spreading direction), thermal stress relief would explain the presence of such faults throughout the entire worldwide ridge system as well as in convecting lava pools (field) and freezing wax (laboratory). It would also explain why transform faults are apparently structurally similar to rift valleys. It would not explain directly why ridge segments are offset; but it is easy to envisage that once a thermal contraction crack appears in a ridge, small random variations in spreading rate on the two sides of the ridge will lead to offsetting.

Membrane stresses, on the other hand, are more obviously relevant to both ocean floors and continents; and it is to a plate comprising both continental and oceanic elements that Oxburgh and Turcotte (*Earth planet. Sci. Lett.*, **22**, 133; 1974) have now applied the concepts they have jointly and separately developed during the past year. The plate is the African plate; and the object of the exercise was to determine whether or not the formation and properties of the East African rift are explicable in terms of membrane tectonics.

The basic principle of membrane tectonics is that the two principal radii of curvature of a small plate at the Earth's surface will increase if the plate moves towards higher latitudes and, conversely, decrease if it moves towards lower latitudes. Thus an unstressed plate (regarded as a thin elastic shell) moving towards the poles will acquire a tension at the edge and a compression in the interior, whereas for a similar plate moving towards the equator the sites of tension and compression will be reversed. The difference between the Earth's polar and equatorial radii of curvature is such that a plate undergoing even a modest change in latitude (a few tens of degrees) will be subject to stresses comparable to the strength of the plate;

and so under the right circumstances large-scale lithospheric fractures may occur.

As interpreted by Oxburgh and Turcotte, the palaeomagnetic evidence suggests that, following a period of relative quiescence lasting most of the Mesozoic, the eastern part of Africa began to move northwards about 100 million years (Myr) ago at a rate of about 0.25° per million years. The western part of Africa, by contrast, began to move much more slowly. Taking into account the drift rate and the size of the African plate as extrapolated backwards throughout the past 100 Myr, Oxburgh and Turcotte calculate that the tensional stress in the centre of a circular plate the size of the African plate (and drifting towards the equator) would be about 135 bar, which seems to be of the same order as the stresses associated with current continental faulting. In other words, it is quite conceivable that the East African rift system was produced by membrane stresses in the lithosphere, developed in response to the rapid latitude change of eastern Africa during the late Cretaceous and early Tertiary—and that is precisely what Oxburgh and Turcotte propose happened.

It follows from this interpretation that the East African rift system is probably a southward-propagating fracture system. There is little direct evidence on the age of the fracture itself; but it is implicit in a propagating fracture system that the onset of rift volcanism will migrate at the same rate as that at which the fracture extends. At most places along the East African rift, the volcanism, once started, has continued to the present; and so the onset of volcanism may be determined from the age of the oldest volcanic rocks available. Such age data from the eastern rift show that the locus of this onset has migrated southwards at a rate of about 0.23° per million years, which is in excellent agreement with the palaeomagnetically-determined northward drift rate of the eastern part of the plate. Numbers apart, however, the advantage of regarding the East African rift as a propagating fracture rather than the locus of a hot spot is that so much more can be explained. The tensional features of the rift are an obvious case in point. Equally, hot spots seem incapable of explaining why volcanism continues at any given point once the plate has passed over the spot; a propagating fracture, on the other hand, would imply continued dilation and thus continued volcanism.

The Oxburgh–Turcotte model is, of course, an oversimplification. The real African plate is not circular, does not remain the same size, and does not undergo a simple change of curvature. Moreover, the unstressed radii of curvature are not constant over the plate but vary from element to element within it. The oceanic lithosphere within the plate is subject to thermal stresses as well as membrane stresses; the location of fracturing within continental lithosphere may be governed partly by previous history of faulting; even mid-plate areas will be affected to some extent by interactions with neighbouring plates; and so on. But these are points of detail, rather than principle, which do not deflect Oxburgh and Turcotte from their view that membrane stress effects are substantially more plausible than hot spot processes.

Internal magnetic field on Mercury?

from Peter J. Smith
Geomagnetism Correspondent

HITHERTO, it has not been considered likely that Mercury possesses an internal magnetic field. It is true that the planet has an unusually high density and thus by implication a large core; but the low rate of rotation would seem to preclude the generation of internal fields by dynamo action. Moreover, unlike Jupiter, Mercury has never produced any evidence of radio emissions which would indicate the presence of radiation belts.

The preliminary data from the Mariner 10 magnetic field survey near Mercury, now recorded by Ness *et al.* (*Science*, **185**, 151; 1974), are thus surprising. For on March 29, 1974 Mariner 10 passed within 704 km of Mercury's surface where it recorded a magnetic field of 98 γ, which is about five times higher than the average interplanetary field in the vicinity. Moreover, both a well developed bow shock wave and a magnetosphere-like region were observed — features comparable to those of the Earth, which has a global field, but completely alien to a body such as the Moon, which does not.

So do these results imply an internal Mercurian field? They certainly imply it, but do not prove it because there are other processes, such as field induction by solar wind interaction, which could just explain the limited data yet available. Another Mercury encounter will be required to settle the matter; but in the meantime Ness and his colleagues interpret the whole evidence as generally favouring the intrinsic field idea. Approximate figures then suggest that the Mercurian dipole moment is about 4×10^{-4} that of the Earth, that the dipole is close to the rotation axis and that it is offset by 0.47 Mercurian radii (not unacceptable in view of the large core).

A dynamo currently active within Mercury would indicate the viability of dynamo action under unexpected and unusual conditions. On the other hand, if the Mercurian field is due to remanent magnetism left from a dynamo now extinct, the implication must be that the planet once rotated much faster than it does now. In any event, internal planetary magnetic fields are rare enough to make the discovery of one within Mercury (if confirmed) a major advance in the exploration of the Solar System.

Ribosome structure and function

from a Correspondent

THE EMBO meeting on ribosome structure (Göteborg, Sweden, June 27-30) was a field day for *Escherichia coli*. The most popular topic was the three-dimensional arrangement of the ribosomal proteins, where several different approaches are being used with considerable success. C. Cantor (Columbia University, New York) has used a fluorescence method in which reconstituted 30S ribosomes are labelled with two different fluorescent dyes on two specific proteins. By varying the proteins labelled, he has been able to classify many pairs of 30S proteins as being close, or far apart in the ribosome.

Experiments with bifunctional protein cross-linking reagents were reported by several delegates, the most impressive being those of R. Traut (University of California, Davis) who used the assymetric reagent 4-mercapto-butyrimidate to establish nearly twenty pairs of cross-linkable proteins from both subparticles. This reagent reacts first with ε amino groups, leaving the thiol group free to react with protein sulphydryl groups under oxidising conditions.

G. Stöffler and G. Tischendorf (Max-Planck-Institut, Berlin) have approached the topography question by electron microscopy. Since both subparticles have an assymetric shape, it is possible to localise the positions of proteins by examining the complexes formed with the ribosome by individual protein-specific antibodies. So far twenty 50S and seven 30S proteins have been localised by this technique. H. Zeichhardt (Max-Planck-Institut, Berlin) used specific antibodies to examine the subparticle interface; although many

Fabs would inhibit the reassociation of subparticles, only one protein (L19) was inaccessible to antibodies in the 70S particle itself. H. Roth (Max-Planck-Institut, Berlin) has isolated more than twenty ribonucleoprotein fragments from LiCl cores of the 50S subparticle by digestion with nuclease. The fragments, separated on sucrose gradients, contained from two to twenty-eight proteins. With a few notable exceptions, the data from these various sources were in good agreement, and two topographical models of the 30S proteins were presented by R. Traut and A. Bollen (Université de Bruxelles).

On the RNA front, C. Ehresmann (Institut de Biologie Moléculaire, Strasbourg) reported the latest improvements on the primary sequence of 16S RNA, and C. Branlant (Institut de Biologie Moléculaire, Strasbourg) described some binding sites of 50S proteins on the 23S RNA, in particular the complex formed between 5S RNA (with L5, L18 and L25) and 23S RNA in the presence of L2. As far as primary structure of individual proteins is concerned, it seems that L7 and L12 have been strongly conserved during evolution; two acidic proteins from rat liver were shown to be related immunologically to L7/L12 (G. Stöffler and I. Wool, Max-Planck-Institut, Berlin), and the corresponding proteins from the halophile *Halobacterium cutirubrum* showed large homologous sequences (A. Matheson, National Research Council of Canada, Ottawa). S5 has again turned up as an altered protein in several ribosomal mutants, notably in revertants from valyl-tRNA synthetase mutants described by H. Wittman (Max-Planck-Institut, Berlin). K. Isono (Max - Planck - Institut, Berlin) also found alterations in the *Bacillus stearothermophilus* protein corresponding to S5 (as well as S12) in streptomycin-resistant mutants.

On the functional side, S12 was shown by A. Spirin (Academy of Sciences of the USSR, Poustchino) to exert a negative control over non-enzymatic translocation, since particles reconstituted without S12 showed increased activity in this assay. With the 50S particle, K. Nierhaus (Max-Planck-Institut, Berlin) found that L11 and L16 are the proteins responsible for chloramphenicol binding. L6 is also important for the chloramphenicol effect, but does not actually bind the antibiotic. L11 binds thiostrepton (J. Gordon and J. Highland, Friedrich Miescher Institut, Basel), and is also involved together with L18 at the peptidyl transferase centre, as was shown by C. Cantor with a peptidyl tRNA photo-affinity label. E. Küchler (University of Vienna) also used a photo-affinity label to demonstrate that peptidyl tRNA can be bound to 23S RNA near to the binding site of the 5S RNA-protein complex. Evidence for conformational changes during protein synthesis came from D. Vasquez (Instituto de Biologia Celular, Madrid), who found that the antibiotic anisomycin binds to eukaryotic ribosomes with different affinities according to the functional state of the particles.

Looking at the more intimate details of tRNA binding, M. Sprinzl (Max-Planck-Institut, Göttingen) used suitably modified tRNA analogues to demonstrate that tRNA synthetases attach the amino acids to the 2′-hydroxyl position of the terminal ribose. Elongation factor T_u also recognises only a 2′-acylated tRNA. Peptidyl transfer can be effected to either a 2′- or 3′-acylated tRNA at the A site, but only from a 3′-acylated moiety at the P site. V. Erdmann (Max-Planck-Institut, Berlin) showed that the oligonucleotide TψCG can be used instead of a complete de-acylated tRNA to stimulate magic spot synthesis.

Last but by no means least, M. Nomura (University of Wisconsin, Madison) reported some elegant experiments with λ phages containing various amounts of the genetic material in the aroEstrA region of the *E. coli* genome. Using a DNA-dependent protein-synthesising system, he found that at least ten 30S and fourteen 50S proteins are encoded within this segment of DNA. The technique offers the possibility of detailed mapping of the ribosomal protein genes.

How microorganisms damage plants

from a Correspondent

In plant, as in animal pathology much research effort has been directed towards investigating the mechanisms by which pathogens damage their hosts. One of the most important advances of recent years has been the realisation that in a small, although by no means unimportant group of plant pathogens, the ability to produce toxin is the major factor determining virulence towards specific hosts. The most convincing experiment to demonstrate this relationship was performed by Scheffer and his associates (*Phytopathology*, **57**, 1288; 1967). They crossed two closely related toxin-producing fungi *Cochliobolus victoriae* and *C. carbonum*, pathogens respectively of oats and maize. F_1 offspring segregated in a 1:1:1:1:1 ratio for the abilities to produce *C. victoriae* toxin, *C. carbonum* toxin, both toxins or no toxin. These progeny were respectively pathogenic on oats, maize, both oats and maize or neither host.

Attempts to elucidate the mechanism of action of these host-specific toxins have been stimulated by the knowledge that these toxins comprise some of the most potent biologically active substances known. Root growth of susceptible oat seedlings treated with *C. victoriae* toxin is prevented by as little as 0.0002 μg toxin ml^{-1}, a potency approaching that of the more active clostridial toxins. In susceptible plants almost any metabolic system that one chooses to examine is deranged by toxin treatment. Many of these changes are undoubtedly secondary effects and evidence is accumulating that the primary site of action of at least some toxins resides in the plasmalemma. Supporting evidence for this hypothesis comes from the findings that toxin treatment of susceptible host cells leads to a rapid leakage of intracellular contents (Scheffer and Samaddar,, *Rec. Adv. Phytochem.*, **3**, 123; 1970; Keck and Hodges, *Phytopathology*, **63**, 226; 1973), to a rapid depolarisation of the plasmamembrane potential (Novacky and Hanchey, *Physiol. Pl. Path.*, **4**, 161; 1974) and also results in an inhibition of solute uptake and failure of the protoplasts to plasmolyse in hypertonic solutions.

Even more convincing evidence has come from the studies of Strobel and his associates (*J. biol. Chem.*, **248**, 1321; 1973; *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1693; 1973; *ibid.*, **71**, 1413; 1974) on the eyespot disease of sugar cane caused by *Helminthosporium sacchari*. The toxin produced by this fungus seems to be a primary determinant of disease and has been characterised as 2-hydroxy-cyclopropyl-α-D-galactopyranoside. Strobel has found that sensitivity to the toxin is correlated with the presence of a specific toxin-binding receptor protein in the plasmalemma of susceptible cells. Those cells resistant to the toxin possess an immunologically identical protein which does not bind the toxin. The specific proteins from resistant and susceptible cells have similar molecular weights, both consist of four subunits but differ slightly in electrophoretic mobility and with respect to four different amino acid residues.

This is the first positive identification of a specific chemical factor associated with disease susceptibility in a higher plant, an association long suspected on theoretical and genetical grounds. Although these reports establish the presence of a toxin-binding protein within the plasmalemma, much remains to be discovered as to the mechanism by which the toxin–protein interaction destroys the normal functioning of the membrane. These reports also illustrate another basic similarity between the functioning of animal and plant cells and suggest that the study of plant cell surfaces should prove useful.

The ageing, growth and death of cells

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The ageing and death of cells in higher plants and higher animals is discussed in relation to cellular rejuvenation by growth and division.

No cell is immortal. If a cell grows and divides, it becomes two cells; if it does not divide, sooner or later it dies. Multicellular organisms are not aggregates of cells in a state of exponential growth and division. Some cells divide, but most differentiate and do not undergo further division. Here I shall discuss the ageing and death of cells in vascular plants and vertebrate animals in an attempt to explore the significance of these processes in relation to growth and development, both normal and abnormal.

It is often convenient to think of cells or living organisms as maintaining a more or less steady state; but it is also easy to forget that this is an approximation, an abstraction which, if realised, would confer on cells and on organisms the doubtful blessings of eternal life and eternal youth. The realities of growth, development, ageing and death cannot be understood in terms of steady state concepts. They are directional and irreversible changes in time.

Some cells die as they differentiate, for example xylem cells in plants and keratinised epidermal cells in animals; some, such as phloem sieve tubes in plants and red blood cells in mammals, lose their nuclei; others which retain their nuclei may lose their ability to divide—as do most nerve cells. But even cells which retain their ability to divide will die if they do not do so; they senesce.

Any general hypothesis of cellular ageing must not only explain cellular ageing itself, but also the way in which some cell lines do not senesce and die out. The germ cell line is continuous from generation to generation (also many plants can be propagated vegetatively indefinitely) and some cell lines derived from plant or animal tissues can be propagated *in vitro* for an indefinite number of generations.

General hypotheses of ageing based on genetic mutation face two difficulties. First, in explaining the universality of the processes of ageing in non dividing cells in terms of a lethal accumulation of harmful mutations and, second, in accounting for the facts of sexual and vegetative reproduction, which show that mutations do not accumulate to a lethal extent in all cells. The alternative to a genetic-mutation hypothesis of ageing is some sort of 'cytoplasmic' hypothesis, the most recent and best known of which is Orgel's 'error catastrophe' hypothesis, which postulates that an accumulation of errors in protein synthesis leads to a positive feedback of error as the enzymes involved in protein synthesis themselves develop errors and thus produce more defective proteins[1]. For this hypothesis to account for the continuity of the germ line and the indefinite propagation of 'permanent cell lines' *in vitro* it is necessary to postulate a process of 'cellular selection' whereby error-containing cells are selected out[2].

Neither the genetic-mutation hypothesis[3] nor the protein synthesis 'error catastrophe' hypothesis[2] of ageing are supported by sufficient evidence to rule out the possibility that cellular ageing may be explicable in terms of the accumulation of cytoplasmic breakdown products, some of which might be deleterious to the cell if they accumulated sufficiently. In all actively metabolising cells, there is a turnover of cytoplasmic constituents such as proteins and membrane lipids. More is known about their synthesis than about their breakdown *in vivo*; while some of them may be broken down completely, others may be broken down only partially or not at all. They must therefore accumulate.

Lipid peroxidation

One example of such an accumulation is provided by the 'age pigment' or lipofuscin granules which accumulate in an age-dependent way in the cells of many mammalian tissues[4]. The lipofuscin material contains lipid and protein and may be formed in autophagosomal vesicles, for example during the digestion of mitochondria; haem groups, released as the cytochromes are degraded, may catalyse the peroxidation of unsaturated lipids in the degenerating mitochondrial membranes[5] which may cross link with each other and with denatured proteins[6]. The cells seem to be unable to destroy these cross-linked polymers. Lipofuscin granules do not seem to damage cells directly except when they accumulate, as they do in certain diseases, to such an extent that they mechanically interfere with the structure and functions of the cell[7].

Lipid peroxidation does not always result in the formation of microscopically visible lipofuscin granules, nor is it confined to autophagosomal vesicles; it occurs in all functional cell membranes, including the surface membrane. Once the peroxidation of unsaturated lipids is initiated, by haem groups, Fe^{2+} ions and other simple catalysts in the presence of oxygen, it takes place by a free radical chain reaction. It can be inhibited by lipid-soluble antioxidants such as vitamin E and accelerated by vitamin E deficiency, ionising radiation, chloroform and ethanol poisoning and hyperbaric treatments[8,9], which can cause irreversible damage to cells.

The peroxidation of lipids within cell membranes is occurring *in vivo* all the time. Some peroxidised lipids may be metabolised[9] but others, perhaps those which are cross linked to other lipids and lipoproteins may not be. The chain reaction of lipid peroxidation may be terminated by the oxidation of other substances which may themselves be damaged and accumulate[10]. Such substances formed within the surface membrane, for example, may accumulate *in situ*; if they are removed from the surface membrane as the membrane is recycled by the invagination of membrane vesicles[11–14] or by other means, some of them might find their way into residual bodies, but they might also be incorporated into intracellular membranes. The formation and accumulation of such substances within the outer and intracellular membranes, for example in the Golgi apparatus, endoplasmic reticulum nuclear membrane and lysosomal membranes, could well be deleterious to normal membrane functioning and could also lead to a positive feedback of damage by further lipid peroxidation, and thus to the senescence and death of the cell. The rate of ageing would be temperature dependent and would also depend on the composition, structure and functions of the cellular membranes, the extra- and intracellular environments, antioxidant levels and so on. Thus, different types of cells would

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age at different rates but, according to this hypothesis, all cells would be ageing to a greater or lesser extent all the time; all cells would be heading towards senescence and death.

The elimination of membranous material from cells might enable the ageing process to be retarded and there are a few examples of the shedding of membranes by cells which I will discuss further. But, in general, the only way in which cells could avoid their otherwise inevitable mortality would be by growing and dividing, thus diluting the accumulated breakdown products. Although lipid peroxidation may be the most important cause of the formation of such substances, the following general considerations could apply to any deleterious substances which accumulate with age.

Growth and division of cells

An artificially simple case is provided by cells dividing symmetrically with a fixed generation time if these accumulate deleterious breakdown products linearly with time, an amount, x, being formed per cell generation time (Fig. 1). Successive generations contain more of the accumulated breakdown products but the increments become smaller and smaller. If the rate of accumulation is not linear, but proportional to the amount already accumulated, the content per cell will increase exponentially; and if there is a progressive lengthening of the cell generation time, there will be a greater accumulation within individual cells in succeeding generations. With either or both of these assumptions, it can be seen that the whole population will undergo senescence and sooner or later die out.

But another type of cell division is possible, an asymmetrical division in which one of the daughter cells receives all or most of the accumulated breakdown products (becoming more 'mortal') while the other is rejuvenated, receiving little or none. The more 'mortal' of the daughter cells might die or differentiate directly, or it might divide again unequally, producing a rejuvenated cell and a cell even more 'mortal' than itself, or it might undergo one or more sequential symmetrical divisions (as discussed above) to produce a population of cells which sooner or later die (unless they can undergo further asymmetrical divisions to produce rejuvenated cells).

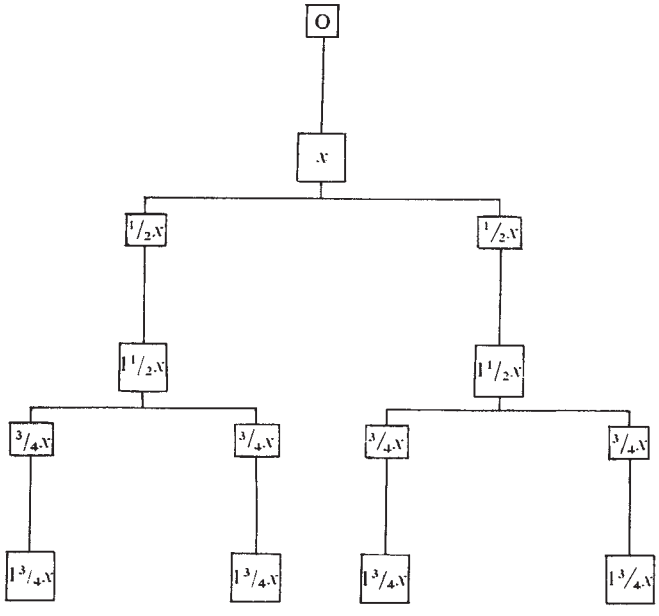


Fig. 1 Cells dividing symmetrically with a fixed generation time showing the accumulation of deleterious breakdown products linearly with time, an amount, x, being formed per cell generation time.

I shall now consider a few aspects of the growth and development of higher plants and higher animals in the light of these ideas. Dicotyledonous trees illustrate the pattern of indefinite growth that is characteristic of plants. (There are of course plants, such as herbaceous annuals, which die after they have flowered. But annuals are capable of growing for much longer than their normal life-span if they are prevented from flowering, indicating that they die because they flower and not because of an innate inability to go on growing[15].) The life span of trees is limited by a variety of mechanical factors, but cuttings taken from old trees can give rise to healthy young trees, and this process can be repeated indefinitely. The growing points of the tree, the apical meristems, remain perpetually young.

Cell divisions within the apical meristems of the shoots give rise to daughter cells with different fates: some remain meristematic, others give rise to the differentiated structures of the stems and the leaves. Some of these cells die as they differentiate into vascular tissues and fibres, others, for example the leaf mesophyll and pith parenchyma, remain alive for some time, but, unless they are stimulated to divide again in a regenerative response to wounding or damage, they eventually die. The leaves senesce and fall from the tree; the pith breaks down. The root meristems give rise to the primary tissues of the root which, apart from those which divide to produce further root meristems, sooner or later die. In secondarily thickening stems the divisions of the cambial cells give rise to cells which die as they differentiate into xylem or undergo further asymmetrical divisions to produce phloem companion cells and sieve tubes. These cells eventually die and are sloughed off in the bark. Cell divisions in the cork cambium give rise to cork cells which die as they differentiate; divisions of the root cap initials give rise to root cap cells which die and are sloughed off. Thus, in the various meristems of the plant the continued growth and continued rejuvenation of the meristems is associated with the production of cells which die during or after differentiation.

Vertebrates

Vertebrates, unlike trees, do not go on growing indefinitely, nor can they be propagated vegetatively. At first, fertilised eggs undergo cleavages which rapidly increase the number of cells, but this rate of increase of cell number declines progressively as the animal develops, and as cells and tissues differentiate[16]. Throughout the development of the embryo many tissues and groups of cells regress and die[17,18]. Some of these cell deaths are associated with tissue differentiation[19], some occur during morphogenetic processes[20], and others may represent the regression of phylogenetically vestigial structures[17], but the significance of other cell deaths is obscure[17]. As the animal develops, the cells of some tissues, such as nerve and muscle, differentiate and to a large extent lose the ability to undergo further division. Some of these cells die as the animal grows older and are not replaced[21,22] but in the adult animal a number of other tissues continue to grow, for example the epidermis, the intestinal lining, the liver and blood cells continue to be formed. In all these examples the production of new cells is offset by cell death. Cell divisions in the basal layers of the mammalian epidermis give rise to daughter cells which remain in the basal layers and divide again, and other daughter cells which differentiate and keratinise, dying as they do so. Cell divisions in the crypts of the intestinal villi replenish the population of crypt cells capable of further division and produce other daughter cells which move up the villi where they die and are sloughed off[23]. Asymmetrical divisions of the early precursors of all cells of the blood occur throughout life and give rise to further precursor cells as well as to the maturing and mature cells of the blood, all of which have a limited life span. During

the formation of red blood cells[24] and granulocytes[25] in the bone marrow, and lymphocytes in the thymus[26], considerable numbers of cells die *in situ* soon after they are formed. The reasons for this 'ineffective' erythropoiesis, granulopoiesis and lymphopoiesis are unknown.

The mortality of at least some of the cells which die in developing animal embryos and in mature animals may represent the price that is paid for the rejuvenation of other cells which continue to grow and divide. But unfortunately too little is known about cell lineages in animals, especially in embryos, for it to be possible to decide how general is the phenomenon of asymmetrical cell divisions giving rise to daughter cells of unequal mortality. The recognition of this pattern is complicated by the fact that by no means all cell death takes place as a result of cellular senescence. Some cells die as they differentiate and others may die because they find themselves in the wrong places at the wrong times[19]. Cell deaths may be controlled chemically, for example by steroid hormones: the injection of glucocorticoids can cause large numbers of lymphocytes to die[27], the regression of Mullerian and Wolffian ducts is controlled by androgens and oestrogens[19,28] and the regression of the lining of the female genital tract is under the control of oestrogens[28]. But, under the hypothesis that asymmetrical cell divisions lead to a rejuvenation of 'meristematic' daughter cells at the price of the increased mortality of their sister cells, it does not matter whether the latter die as a result of senescence, or whether they die as they differentiate or for any other reason.

Sexual reproduction

In the sexual reproduction of both higher plants and higher animals almost all the cytoplasm from which the embryo and the new organism develops is provided by the egg. In both cases, the egg cells are formed as a result of asymmetrical divisions of the egg mother cell. In the great majority of higher plants, the meiotic divisions of the egg mother cell produce four cells, three of which die. The fourth undergoes further divisions to produce the cells of the embryo sac, most of which die before or shortly after fertilisation. In some species, one or more of the three sister cells of the cell which gives rise to the egg may undergo further division to produce short-lived embryo sac cells[29]. In animals the first and second meiotic divisions of the egg mother cell give rise to the first and second polar bodies, which regress and die[30,31].

It is particularly striking that in both plants and animals, only one of the progeny of the egg mother cell gives rise to an egg while the sister cells die (or if they divide give rise to short-lived progeny). By contrast, there is no comparable cell loss in male gametogenesis associated with the meiotic divisions of the pollen mother cells and spermatogonia.

The many examples in both higher plants and higher animals (and many more can be found in the lower plants and lower animals) of the production of rejuvenated meristematic, stem or egg cells by asymmetrical divisions do not of course prove that these divisions involve an asymmetrical distribution of deleterious breakdown products; but the available facts appear to be consistent with this hypothesis.

Loss of membranous material by animal cells

If the accumulation of deleterious breakdown products of membrane lipids is one of the causes of cellular senescence, the loss of membranous material might be of considerable importance in enabling cells to rid themselves of such substances. The shedding of membranous material by living cells does not seem to be of common occurrence but can take place in mammalian cells as follows.

First, in apocrine secretions part of the cell membrane is lost. The best example, and the only one for which conclusive ultrastructural evidence exists, is in the secretion of lipid droplets by the cells of lactating mammary glands. The secreted lipid droplets are surrounded by a unit membrane derived in part from the surface membrane and in part from Golgi vesicle membranes[32].

Second, membrane-bounded vesicles of cytoplasm can break away from mammalian macrophages both *in vitro* and *in vivo*[33]. This process, known as clasmotosis, is of unknown significance. Lymphocytes which are activated in immunological reactions or as a result of phytohaemagglutinin stimulation form 'tails' (uropods) which can bleb off vesiculated buds *in vivo* and *in vitro*[34]. Again, the significance of this process is unknown. Clasmotosis is also frequently observed in cultures of fibroblasts.

Third, many types of animal viruses are budded off from host cells in membrane-bounded vesicles. The protein in the membrane of the vesicles is largely viral, at least in the case of RNA tumour viruses, but the lipids are derived from the host cell membrane[35]. Viral particles bounded by membrane are also budded off from the cells of a number of spontaneously cancerous tissues[36] and from many of the cell strains and permanent cell lines which are commonly cultured in laboratories[37].

Tissue cultures

Many plant callus cultures can be grown indefinitely *in vitro*. During the early stages of the growth of some calluses, an exponential increase in cell number takes place at a rate which suggests that many of the cells may undergo a limited number of sequential symmetrical divisions before the growth rate declines[38,39] but in most plant tissue cultures the rate of increase of cell number is more or less linear for most of the growth period[39,40]. Linear growth characteristics would be compatible with a meristematic pattern of cell division such that some daughter cells continue to grow and divide while their sister cells age and sooner or later die. Unfortunately nothing is known in detail about cell lineages within these cultures, nor are there any quantitative data on cell death. Nevertheless, dead and dying cells are by no means uncommon.

'Permanent' mammalian cell lines capable of indefinite propagation *in vitro* can be derived from cancerous tissues and also from cells which have undergone a spontaneous 'transformation' during culture. Diploid fibroblast cultures can be propagated, however, only for a finite number of subculturings, more (up to about 60) if the cells are derived from embryonic tissues, fewer if they are derived from mature organisms[41]. The number of generations through which the cells can be passed before the population senesces and dies out is reduced if the period of time between the subculturings is increased[42]. Fibroblasts of the mouse L strain have been observed to divide symmetrically over six to seven cell generations with a more or less constant generation time[43]; if the cells in the diploid fibroblast cultures also divide symmetrically, deleterious breakdown products might accumulate in the cells of succeeding generations, as discussed above, and account for the senescence of these cultures. It is impossible, however, to make any detailed interpretation of the senescence of these cultures in the absence of quantitative information about the proportions of dividing and nondividing cells, the incidence of cell death, and the extent and significance of clasmotosis within these cultures—or indeed with cultures of 'transformed' and 'permanent' cell lines.

Cancer

Malignancy must not only involve the freeing of cells from the normal controls on their proliferation, but also the avoidance of senescence by at least a part of the cell population. Many animal tumours contain a stem cell or

'meristematic' population which gives rise to daughter cells which may or may not differentiate, but which sooner or later die[44]. There are numerous examples of cell death within cancerous tissues[45–48]. Some of the cell deaths can be explained in terms of an inadequate vascularisation of the tumour tissue, but in most tumours this is by no means the only cause and does not apply at all to leukaemias; many of the cells may die as a result of ageing[44].

Little attention has been paid to the incidence of cell death within cultures of cancerous cells and it is therefore at present impossible to know to what extent the patterns of cell division, ageing and death within these cultures resemble those within *in vivo* cancers. It is sometimes assumed, if only implicitly, that overall exponential growth characteristics of cell cultures mean that there is a homogeneous population of symmetrically dividing cells. This assumption is not justified: a heterogeneous population containing proliferating, nonproliferating and dying cells can also grow exponentially if the proportion of cells that die is constant with time[49,50].

It is conceivable that the loss of membranous material either spontaneously, as in certain types of mammary gland tumours[51], or as a result of the budding off of viruses (such as RNA tumour viruses) could play a significant role in the retardation of cellular senescence in certain types of cancer.

Effects of cell death

Very little is known about the biochemistry of dying cells. Such cells probably release all sorts of proteins, glycoproteins, peptides, amino acids, amino acid breakdown products, nucleic acids and nucleic acid breakdown products, lipids and lipid breakdown products as well as salts and other substances which were sequestered inside the cells.

It has recently been found that in higher plants the hormone auxin (indole-3-acetic acid) is formed as a consequence of cell death as tryptophan, released by proteolysis, is broken down. Dying cells in differentiating vascular tissue, regressing nutritive tissues and so on, are probably the major source of this hormone within the plant[52]. Other plant hormones may also be produced by damaged and dying cells: ethylene from the breakdown of methionine and cytokinins by the hydrolysis of transfer RNA[52]. In higher plants the normal production of hormones as a consequence of cell death and the production of 'wound hormones' by damaged cells can be seen as two aspects of the same phenomenom[52].

Wound and regenerative responses in vertebrates cannot be explained simply in terms of wound hormones, but there is evidence that dying cells release substances that stimulate phagocytosis[53], and affect growth and development in both normal[54,55] and cancerous tissues[56]. And at least some of the cell deaths which occur during normal embryonic development may well result in the production or release of substances involved in the control of differentiation and development.

Dying cells may not only have a chemical effect on neighbouring cells but also a physical effect as cell to cell contacts are broken. Cell deaths within a tissue may also affect the functioning of the tissue as a whole: for example, the death of nerve cells within the brain[22] seems likely to affect pathways or patterns of nervous conduction, perhaps leading to the formation of new pathways or patterns. Such cell deaths could act as a source of random change within the nervous system that might not always be deleterious[57].

So little attention has been paid to the ageing and death of cells during growth and development, both normal and abnormal, that detailed information about these processes is scarce. Where facts are few, speculation can flourish. Most of the speculations advanced in this article could be opposed by alternative speculations, but they illustrate the view that growth and development cannot be understood in isolation from ageing and death. This is by no means an original concept, but at the cellular level it provides a perspective in which many familiar facts take on a new significance and suggests a new approach to familiar problems.

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Recent eruption of Mount Etna

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Mount Etna erupted for the first time in 31 months on January 30, 1974. The eruption, which continued until February 16, was watched closely between January 30 and February 15 by Italian and British members of the Etna Research Programme. This article summarises their observations.

On January 30, 1974 Mount Etna erupted for the first time since the middle of 1971 (ref. 1). Between these eruptions, activity on the volcano had been restricted to strombolian explosions and the extrusion of lava at the bottom of two deep pits within the summit crater. The centre of the new eruption lay 5.75 km to the west of the summit crater, at an altitude of about 1,680 m (grid ref. 938776 on the Monte Minardo 1:25,000 map sheet).

The eruption was first noticed at about dusk on January 30, but it probably started during the afternoon. The first observed activity was the occurrence of strong, almost continuous (30–50 a minute), strombolian explosions which threw bombs to a maximum of 700 m, and rapidly built up a cone. The first erupted lavas (Flow 1) were observed on January 31, as 2–3 m thick aa flows (Fig. 1). At this time the flow front was at the foot of Mt Nuovo (which erupted in 1763). During the next four days, two more lava streams (Flows 2 and 3) were emitted, forming parallel flows which extended between the new cone and that of Mt Nuovo. After three (?) days the explosive activity had decreased in rate to about 25 explosions a minute.

On February 2 (?), two short, thick, viscous flows (Flows 4) erupted from boccas on the eastern side of the cone. They were more than 20 m thick and short lived, and later became largely covered by ash from the cone. Another viscous lava, observed on February 6, probably started erupting on the previous day from a bocca on the northern side of the cone. This flow (Flow 5), an aa flow with a maximum thickness of about 10 m at its front, was advancing on a 70–80 metre front, at a maximum flow rate of 1 m h^{-1}. This rate was, however, evidently reduced, implying that earlier rates must have been much higher to give rise to this flow. The flow stopped on February 7, by which time there was no flowing lava, although the explosion rate in the crater continued at about 14–16 explosions a minute, with maximum projectile heights of about 500 m. On February 8, strong blizzards prevented any observation but by February 9 the violent strombolian activity had changed to occasional explosions which ejected bombs to about 100 m above the crater lip, and there were rumblings within the crater. We considered the magma level in the cone to be very low at this time. On the same day a new aa flow about 800 m long (Flow 7) was discovered emanating from a bocca at the foot of the cone on the south-eastern side of the volcano. The bocca was in a hollow 120 m across with a flat floor containing a small lake of

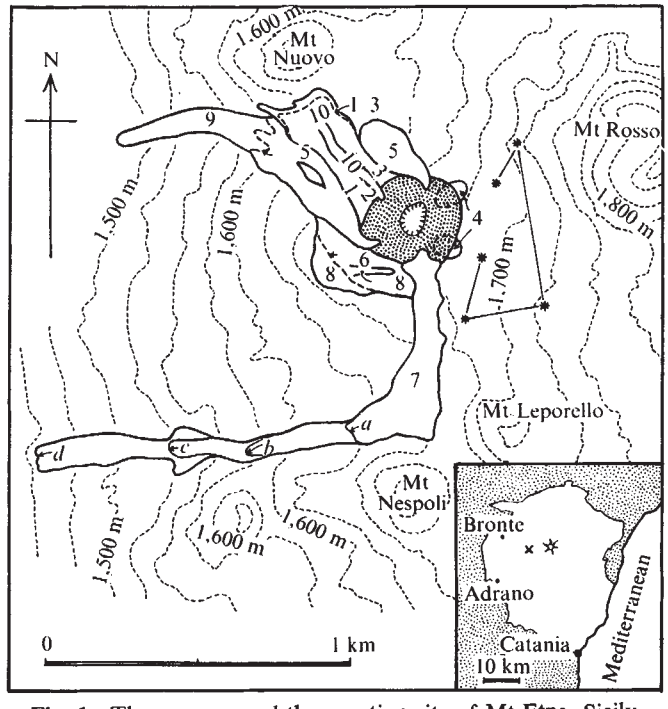


Fig. 1 The area around the eruption site of Mt Etna, Sicily, showing the course of the eruption of January 30 to February 16, 1974. 1–10, Flow numbers (see text); *a*, the extent of Flow 7 on February 9; *b*, February 10; *c*, February 11; *d*, February 15; *, e.d.m. station. Inset: ×, January 1974 eruption, ⁎, summit of Mt Etna.

viscous lava. Lava from this lake was pouring down a short 40° slope towards Mt Leporello, where it turned westwards. The active flow front was about 80 m across and was moving slowly, except on a slope of about 9° down a dirt track, where a flow 3 m across and 1.5 m high advanced at a rate of about 33 cm min^{-1}. The rate of eruption at the bocca was variable and apparently stopped late on February 9, only to restart a few hours later, early the following morning. An examination on February 9 had shown that before this flow, an earlier flow (Flow 6) from the same bocca had flowed around the side of the cone and towards Mt Nuovo, probably on February 8. This flow was about 2–5 m thick.

On February 10, the explosive activity was still discontinuous, with occasional periods of stronger activity (12 explosions every minute) throwing bombs about 150 m above the lip of the cone. The extrusion of lava was also irregular and late in the day the flow front was hardly moving, although several new lobes had developed along the southern side. By the morning of February 11, the lava had moved another 250 m and there were only occasional explosions in the crater. Most of the ejected material was derived from collapses of the crater walls. A gas jet formed at the bocca of Flow 7 and sublimates were deposited. The flow front was advancing at about 30 cm min^{-1}, but it had slowed down to 1 m h^{-1} on an 80-m front by February 13. On that day it was discovered that a bocca had opened on the south side of the cone. Lava (Flow 8) from this bocca was flowing around the foot of the cone, towards the west. By 1700h LT, Flow 7 had stopped and Flow 8 was flowing at a rate of 0.3 m min^{-1}. A new bocca (1–2 m across) had opened to the west of the cone, extruding lava (Flow 9) which flowed at a rate of 3 m min^{-1}. Another bocca (Flow 10) to the north-north-west of the cone had also apparently opened but we did not visit this until the next day. Weak explosions continued in the crater, and there were strong rumblings with gas explosions which ejected a little pyroclastic material. Ground shocks could be felt up to 900 m from the cone. By February 14, Flow 8 had stopped, but Flow 9 was still erupting, flowing at about 2.5 m min^{-1} in a flow 3–5 m wide. On February 14 and 15, the inner walls of the cone suffered major collapses. On the latter day, we observed radial fractures on the southern and western flanks of the cone. The rate of flow in Flow 9 was about 4 m min^{-1} and Flow 10 was erupting at a rate of 2 m min^{-1} and was 1.5 m wide. By February 17, the activity had stopped. Twenty-three days later a further period of activity began in the same area, about 200 m to the south-west of the centre of activity which we had been observing.

We computed the amounts of lava which were erupted during the February period of activity: between January 30 and February 7, 1.0×10^6 m^3; February 8 and 9, 1.0×10^6 m^3; February 10–15, 0.35×10^6 m^3. This is a total of 2.35×10^6 m^3, giving an average rate of eruption of about 1.5 m^3 s^{-1}, although on February 8 and 9 the rate may have been more than 6 m^3 s^{-1} for a short period, thus lowering the level of magma in the cone and reducing the strength of the explosions.

Viscosities inferred from the rate of flow in channels of Flow 6 were about 1.8×10^5 poise 50 m downstream from the bocca, where lava surface temperatures were about 980° C (measured with an optical pyrometer), and about 1.0×10^6 poise near the flow front, where lava surface temperatures of 962° C were measured (using an optical pyrometer and a thermocouple). In the boccas of Flow 6 and later flows, maximum temperatures of 1,060° C were measured with a thermocouple inserted to 20 cm.

Activity at the central crater

We visited the Central Crater at the summit of Etna (3,200 m) on February 12. There are two pits within the summit crater[2], but only one, the Bocca Nuova, showed any significant activity—the spasmodic expulsion of clouds of very fine, brown ash, accompanied by muffled explosions, which were estimated to originate from a depth of several hundred metres. Quiet emission of fume was occurring at both the Chasm and the 1964 Crater, but the NE Crater remained inactive.

Table 1 Analyses of lavas and bombs*

	JG/E1†	JG/E6‡
SiO_2	47.75	47.74
TiO_2	1.81	1.79
Al_2O_3	17.25	17.25
Fe_2O_3	5.24	5.16
FeO	5.54	5.62
MnO	0.22	0.21
MgO	5.51	5.49
CaO	10.45	10.46
Na_2O	3.17	3.17
K_2O	1.65	1.65
P_2O_5	0.34	0.37
CO_2	0.16	0.16
H_2O (total)	0.48	0.39
Total	99.57	99.46

* Analysis by A. C. S. Smith (University College, London). Both analyses are the mean of duplicate analyses using the atomic absorption method.

† Fresh bomb ejected on February 7, and partially quenched in snow.

‡ Quenched lava from the front of Flow 7 (February 11).

The vigorous strombolian activity which characterised the Chasm and the Bocca Nuova during parts of 1973 left a deposit of ash and bombs near the Chasm. This activity, which reached its culmination in November, seems to have raised the floor of the Chasm from about 150 m to about 75 m below its lowest lip. Since then, however, there has been a lowering of the Chasm floor. It now falls in a series of steps produced by arcuate fractures, towards a deep, central pit, 50 m across, from which most of the gas emerges.

This seems to indicate that there has been a drop in magma level or pressure on the Chasm Bocca Nuova conduit system, possibly connected with a movement of magma away from there, and into the western side of the volcano.

Electromagnetic distance measurements (e.d.m.), on the southern side of the volcano, at a height of 2,000 m, indicate a significant amount of deflation of the volcano since July 1973. A quantitative assessment of the role played by the central magma conduit system during the eruption described here must, however, wait till the summit e.d.m. network can be measured later in 1974.

A small e.d.m. network was set up on the eastern side of the eruptive site (Fig. 1) and repeatedly measured from February 10 to February 15. There were no movements recorded above the level of error of the measurements, (± 8 mm, Hewlett-Packard 3800 B).

Eruption type

Compared with known Etnaean eruptions, this eruption had lower rates of effusion, a high viscosity, greater explosivity, possibly lower temperatures and a higher crystallinity of the lavas on extrusion. Ancient eruptive cones with small lava flows nearby suggest that, locally, this type of activity has been common.

Rittmann[3] has classified types of eruption on Etna to include: lateral eruptions, which occur because magma migrates from the central conduit below the summit crater to fissures on the lower flanks; and eccentric eruptions, supplied independently of the central magma conduit. The differences between the eruption described here and the clearly lateral eruptions (for example, the 1971 eruption),

considered together with the seismic activity and the differences in petrology between the new lavas and those present in the Central Crater a few months before the eruption, suggest that this new eruption is fed by a conduit that is not related to the central conduit (eccentric eruption). On the other hand, Guest[4] noted a rise in fumarole temperatures on the western side of the summit crater in 1972 (and in 1973), and predicted a westward migration of magma from the central conduit. Furthermore, the summit pits were active up to a few days before the latest eruption, but they became inactive after the new outbreak, except for deep explosions in the Bocca Nuova. This would be expected for a lateral eruption, although it does not necessarily preclude an eccentric eruption. Unfortunately, heavy snow and bad weather conditions prevented distance measuring at the summit. Further work on the petrology and chemistry (Table 1) of the lavas, together with geodetic studies, will help to determine the origin of the eruption.

Drs R. Romano and L. Villari of the Instituto Internazionale di Vulcanologia, Catania made available their observations and provided help in the field.

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Androgen transport and receptor mechanisms in testis and epididymis

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To influence spermatogenesis and sperm maturation, androgen has to be transported from the Leydig cells to the germinal epithelium and epididymis. This article describes extracellular transport and intracellular receptor mechanisms for androgen.

SPERMATOGENESIS is an androgen dependent process which can be maintained in hypophysectomised rats by the administration of either tesosterone[1] or 5α-dihydrotestosterone[2]. Gonadotrophins may exert their effects on spermatogenesis indirectly by way of androgens. The sole action of luteinising hormone (LH) seems to result from the stimulation of testosterone synthesis by the interstitial Leydig cells. Follicle stimulating hormone (FSH) is necessary for the full maintenance of spermatogenesis, but its effects can be mimicked by androgens and blocked by the 'anti-androgen', cyproterone, indicating that its action is serially linked with androgen[1] or that androgen is the mediator of FSH action.

Androgen binding protein

An androgen binding protein (ABP) with high affinity for 5α-dihydrotestosterone (DHT) ($K_a = 1.25 \times 10^9\ M^{-1}$) and testosterone ($K_a = 0.5 \times 10^9\ M^{-1}$) has been demonstrated in 105,000*g* supernatants of rat testis and epididymis[5–10]. ABP is produced in the testis, secreted into testicular fluid and carried to the epididymis by the efferent ducts[9,10]. The cencentration of ABP in efferent duct fluid (EDF) ($4–8 \times 10^{-8}$ M) is sufficient to bind testosterone and (or) DHT in a concentration of 13–26 ng ml^{-1}, assuming one binding site per molecule ABP (ref. 10). Ligation of efferent ducts causes ABP to disappear completely from caput epididymidis supernatant within 3 d, suggesting that the testis is the only source of ABP in the epididymis[9]. ABP must be produced within the seminiferous tubules, since it is present in EDF and is absent from testicular lymph; also, the presence of ABP in testis supernatants following complete destruction of the germ cells by X-radiation or chemicals, like nitrofurazon, suggests that ABP is formed by Sertoli cells (Hagenäs *et al.*, unpublished observations). It seems that ABP might have an important function in the transport of testicular androgen to the germinal epithelium as well as to androgen-dependent epithelial cells in the epididymis.

ABP in efferent duct fluid and supernatants of testis and epididymis

Concentrations of ABP were measured in testis, efferent duct fluid (EDF) and segments of the epididymis from adult rats. Testis and epididymis segments were homogenised separately at 4° C in three volumes of 50 mM Tris HCl, *p*H 7.4, containing 1.5 mM EDTA, 0.5 mM 2-mercaptoethanol, and 10% glycerol. Homogenates were centrifuged at 105,000*g* for 60 min. Supernatants and EDF were extracted with charcoal (1 mg per mg protein) overnight at 0° C, to remove endogenous steroids and the charcoal removed by centrifugation. Total protein was measured in each sample by the method of Lowry *et al*[11].

Binding capacity of ABP was measured by 'steady state' polyacrylamide gel electrophoresis (SS-PAGE)[12]. By this method, 1,2-^{3}H-5α-dihydrotestosterone (^{3}H-DHT) is dissolved in the acrylamide solution before it polymerises. ^{3}H-DHT is thus distributed uniformly in the polymerised gel and remains stationary in the electrophoretic field until

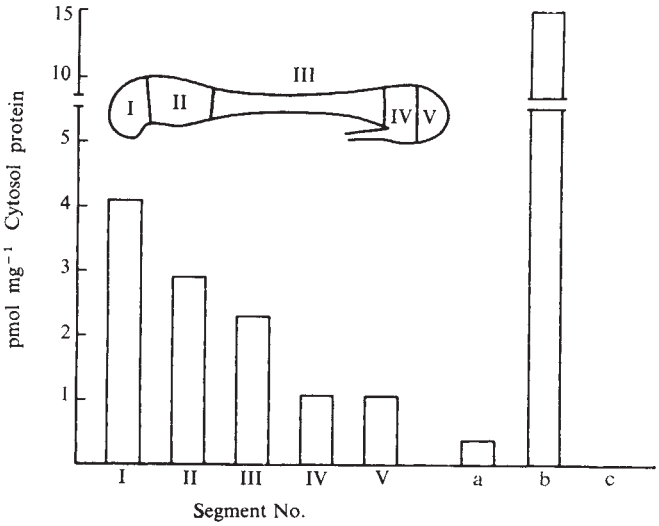


Fig. 1 Concentrations of ABP in 105,000*g* supernatants prepared from segments of rat epididymis in comparison with rat testis (a), efferent duct fluid (EDF) (b), and serum (c). ABP was measured by steady state polyacrylamide gel electrophoresis (SS-PAGE). Samples were adsorbed overnight with dextran coated charcoal at 0° C (1 mg Norit A mg^{-1} protein). Charcoal was removed by centrifugation twice at 10,000*g* in a Beckman microfuge, and samples of 100 µl were layered onto 6.5% acrylamide gels (5 × 70 mm) containing 10% glycerol, to which 2 nM ^{3}H-DHT had been added before polymerisation. Electrophoresis was run at 0° C for 2 h, and 2 mAmp per tube was applied. Following electrophoresis, the gels were transversely sliced into 2.3 mm segments which are placed directly into counting vials with 10 ml toluene scintillation fluid. After standing overnight at room temperature, more than 98% of the radioactivity was extracted into the toluene. Steady state between bound and free ^{3}H-DHT is obtained when the level of radioactivity in front of the ABP peak is identical to that behind the peak. At steady state the amount of ^{3}H-DHT in the peak is a linear function of the total amount of binding protein (ABP) present in the sample: Total ABP = Peak ABP(K_D/[H] + 1) where K_D is the equilibrium constant of dissociation and [H] is the concentration of the ^{3}H-DHT in the gel.

it is bound by protein moving through the gel. After electrophoresis, the gels are sliced and the radioactivity is measured in each slice. A steady state between bound and free radioactivity is reached when the level of free radioactivity behind the binding protein peak equals the level in front. The concentration of binding protein can then be determined (assuming one binding site per protein molecule).

The concentration of ABP in EDF expressed as ^{3}H-DHT binding capacity at saturation (pmol per mg protein) was much higher than in testicular and epididymal supernatants (Fig. 1). There was sufficient ABP in EDF to account for all the ABP measured in supernatants of testis and epididymis. Since the primary fluid from the seminiferous tubules becomes diluted before reaching the rete testis[13], the concentration of ABP surrounding the germinal epithelium might be even higher than in EDF. The relatively large amount of ABP in caput epididymidis supernatant compared with testis supernatant, probably results from absorption of water as the testicular fluid passes through the efferent ducts and the first segment of caput[14], and may also reflect a larger proportion of luminal fluid per unit of tissue weight. ABP concentration in epididymis supernatants decreases from segments I to V (Fig. 1). Further, the amount of ABP leaving the epididymis through the vas deferens is much less than that which enters into the caput, suggesting that the ^{3}H-DHT binding activity of ABP is destroyed during passage through the epididymis. Although the exact role of ABP in the caput epididymidis is not yet known, it probably concentrates large amounts of androgen in the lumen close to the spermatozoa and surrounding epithelial cells. The concentration of endogenous DHT in the caput epididymidis of rats (40–60 ng per g tissue)[15] is several times higher than in any other body tissues so far examined, and is very similar to the concentration of ABP.

ABP production is stimulated by FSH

To establish the relationship between the production of ABP and maturational changes in the testis, ABP was measured in rats of various ages. At 1 and 2 weeks of age, testis and epididymis contained negligible amounts of ABP; at 3–4 weeks, however, increasing amounts of ABP were detected. After 4 weeks, ABP levels increased rapidly in the caput, reaching a peak at about the time of puberty (8 weeks) and decreasing in the adult to about 50% of the pubertal value[16]. Since the appearance of ABP corresponded with the onset of gonadotrophin secretion in the immature rat[17], the effect of hypophysectomy on the levels of ABP was examined in rats at 5 weeks of age. Three days after hypophysectomy, the concentration of ABP in caput epididymidis supernatants was only about 40% of that in intact littermates, and by the 10th day, ABP had completely disappeared from both testis and epididymis. Evidence that this action of the pituitary is mediated through gonadotrophins, and not some other pituitary factor, was obtained by the injection of a human gonadotrophin preparation, containing both FSH and LH, to adult hypophysectomised animals. FSH-LH administration for 5 d caused a distinct increase of ABP in the testis supernatant (Fig. 2). The ABP concentration was more than 10 times higher than in the hypophysectomised controls and almost twice as high as in the intact littermates. These findings strongly indicate that ABP production is under pituitary control both in immature and adult rats.

Highly purified preparations of human FSH and LH were then used to examine their separate effects on ABP production. In 5-week-old intact rats, FSH injected subcutaneously for 10 d markedly increased the concentration of ABP both in the testis and the caput epididymidis, while LH treatment had no significant effect[16]. Similar results were obtained when rats were hypophysectomised at 5 weeks and started on daily injections of FSH 10 d later. After treatment for 10 d, FSH restored ABP levels in testis and epididymis supernatants[16]. When increasing doses of highly purified FSH were injected intramuscularly to immature rats, there was a linear increase in ABP concentration in caput epididymidis at doses between 2–10 µg of FSH

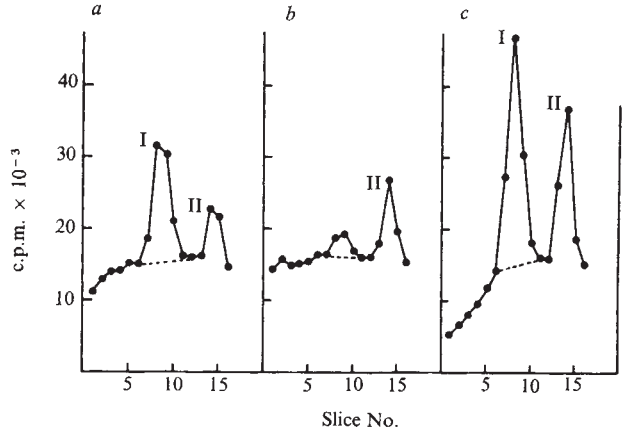


Fig. 2 Effect of FSH + LH treatment on ABP levels in rat testis. Adult rats in groups of four were hypophysectomised for 19 d. From day 20 after hypophysectomy, each animal was injected daily with 75 IU FSH + 25 IU LH or saline only. After treatment for 5 d, the testes were examined for ABP as described in Fig. 1. *a*, Sham operated; *b*, hypophysectomy for 19 d and saline for 5 d; *c*, hypophysectomy for 19 d and FSH + LH for 5 d. I, ABP; II, albumin. ABP, 100% in *a*, 14% in *b* and 157% in *c*.

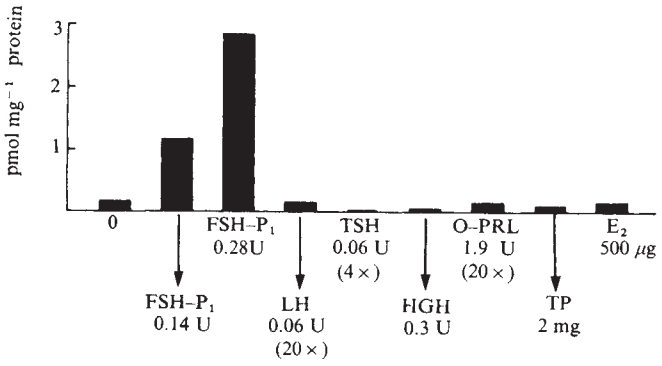


Fig. 3 Specific stimulation of ABP by FSH. Rats in groups of four were hypophysectomised at 28 d of age and injected with 0.14 or 0.28 NIH units FSH-P_1 (total dose). The injections were started on day 30. Parallel groups of animals were injected with high doses of LH, thyroid-stimulating hormone (TSH), ovine prolactin (O-PRL), human growth hormone (HGH), testosterone propionate (TP) and oestradiol-17β (E_2). (20×), Twenty times the maximum contamination of LH or O-PRL in 0.28 units FSH-P_1; (4×), four times the maximum contamination of TSH in 0.28 units FSH-P_1.

(ref. 18). Administration of testosterone propionate (TP) in increasing doses (10–100 μg per 90 g body weight) to intact 50-d-old ('pubertal') rats caused a dose-dependent decrease in ABP concentrations in both testis and epididymidis, indicating that ABP is sensitive to feedback suppression of pituitary gonadotrophins[19].

FSH stimulation of ABP both in intact and hypophysectomised animals is specific, since no increase in ABP could be obtained using other pituitary hormones or sex steroids (Fig. 3). The sensitivity of the ABP response to FSH is comparable with that of the ovarian augmentation test[20,21]. Significant stimulation of ABP in hypophysectomised animals was obtained with as little as 2 IU of FSH (equivalent to about 0.08 NIH units). The ABP response diminished with time after hypophysectomy but was restored by pretreatment with high doses of TP (0.5–16 mg TP per 90 g body weight) (unpublished observations). Similar augmentation of the ABP response to FSH was obtained by pretreatment with LH. The enhanced response to FSH following androgen priming is reflected in increased concentrations of ABP both in testicular and epididymal supernatants; however, it remains to be determined whether TP priming increases Sertoli cell production of ABP or decreases the rate of ABP degradation.

Androgen receptors in epididymal epithelium

After testosterone is taken up by epithelial cells of the epididymis, it is rapidly converted to several metabolites, with DHT and 5α-androstane-3α,17β-diol as major products[22,23]. DHT is bound to intracellular androgen receptors in the cytoplasm[24,25], subsequently transported into the nucleus and bound to nuclear chromatin[22,26]. DHT-receptor complexes can be extracted from epididymal nuclei in 0.4 M KCl and have a sedimentation constant of 3–3.5S (refs 22, 25 and 26).

Epididymal cytoplasmic receptor (CR) has physical properties and steroid specificity very similar to the ventral prostate receptor[24,25,27]; both are highly specific for DHT. They also have similar electrophoretic mobilities by PAGE (R_x = 0.4), similar sedimentation rates by sucrose gradient centrifugation (8S and 4S) and similar elution volumes by Sephadex G200 gel filtration[24–27]. Both are destroyed by heating at 50° C for 30 min and lose binding activity after treatment with sulphydryl blocking agents, such as PCMPS (1 mM)[24,25]. A characteristic feature of ^{3}H-DHT-CR complexes of epididymis and prostate is the very slow rate of dissociation at 0° C ($t_{1/2}$ 0° C > 4 d) (refs 25, 27). This property clearly distinguishes ^{3}H-DHT-CR from ^{3}H-DHT-ABP, which dissociates much more rapidly ($t_{1/2}$ 0° C = 6 min) (ref. 25). The slow rate of dissociation of ^{3}H-DHT-CR is consistent with a receptor function of CR. During the process of translocation from cytoplasm to nucleus, androgen is firmly bound to the receptor and cannot be exchanged by excess of free DHT (ref. 26). The rapid dissociation of DHT from ABP, however, suggests that ABP acts as a carrier protein capable of releasing its androgen to surrounding epithelial cells. A striking difference between ABP and CR was also found in their affinity for cyproterone[25]. Cyproterone acetate inhibited the binding of ^{3}H-DHT to epididymal CR to the same extent as it reduced uptake and binding by epididymal nuclei[25]. Binding of ^{3}H-DHT to ABP on the other hand was not affected by this treatment. These studies indicate that nuclear uptake and binding of DHT are dependent on CR and independent of ABP.

Androgen receptors in the seminiferous tubules

The evidence for androgen action on spermatogenesis prompted studies to discover androgen receptors in rat seminiferous tubules. Immature Sprague-Dawley rats hypophysectomised at 25 or 35 d of age were used. Three to twenty-four days after hypophysectomy, animals in groups of 10 or 20 were eviscerated, functionally hepatectomised[22] and injected intravenously with ^{3}H-testosterone. Whole testes or washed seminiferous tubules were homogenised in three volumes of 50 mM Tris HCl buffer, *p*H 7.4, containing 0.32 M sucrose and 3 mM $MgCl_2$ (TSM buffer); and 105,000*g* supernatants prepared similarly from epididymis and prostate. Binding was examined by PAGE (refs 6 and 8), Sephadex G200 gel filtration[6,24], and by sucrose gradient centrifugation[6,24].

Testicular cytosol fractions contained an androgen–protein complex moving as a symmetrical peak of protein bound radioactivity with an R_x of 0.4 (Fig. 4). The mobility of this androgen–protein complex was different from that

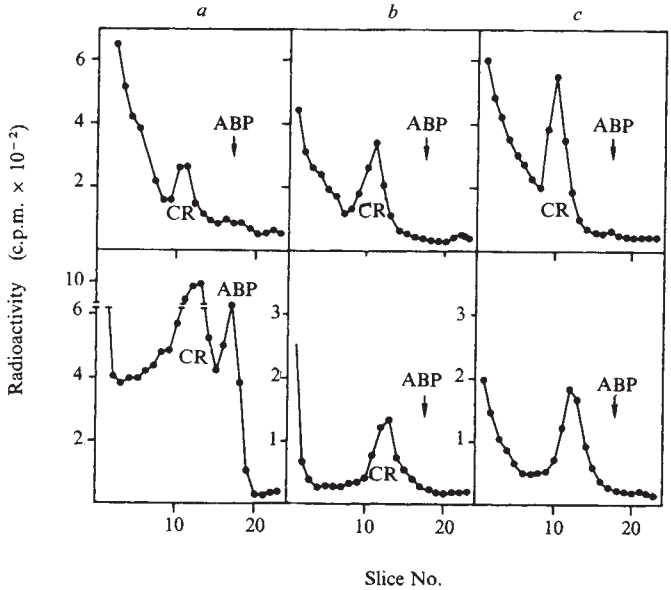


Fig. 4 Cytoplasmic receptors (CR) in testis (upper) and epididymis (lower) different from testicular androgen binding protein (ABP). Sprague-Dawley rats were hypophysectomised at 35 d of age. At different intervals afterwards (*a*, 3; *b*, 10 and *c*, 24 d) animals in groups of 10 or 20 were eviscerated and functionally hepatectomised[22]. Five ml of 5% glucose in water and 200 μg cortisol were given subcutaneously 1 h before the operation. ^{3}H-testosterone (91 Ci mmol^{-1}, 50 μCi) was then injected into the femoral vein, and the animals were killed after 3 h. Testis and epididymis 105,000*g* supernatants (100 μl) were layered over 3.25% acrylamide gels (5×60 mm) containing 0.5% agarose[25]. Electrophoresis was run for 2 h at 0° C and 1.5 mAmp per tube. After electrophoresis, the gels were sliced and counted as in Fig. 1.

of ABP (R_x = 0.71), but very similar to the CR of the epididymis and ventral prostate, which were run simultaneously for comparison. When the seminiferous tubules were separated from interstitial tissue, CR was present in the seminiferous tubular fraction. No androgen binding was detected in cytosol fractions of the interstitial tissue. Radioactivity bound to CR was eluted from the gel and identified by thin layer chromatography (TLC) and crystallisation of the isolated metabolites to constant specific activity. Only testosterone (60%) and DHT (40%) were bound to testicular CR, though these androgens were relatively minor fractions of the total labelled metabolites. Most of the radioactivity in the testis supernatant chromatographed with 5α-androstane-3α,17β-diol and more polar metabolites. In the same animals, the radioactivity bound to the epididymal and prostate CR was more than 90% ^{3}H-DHT.

Gel filtration chromatography of a similar aliquot of testicular supernatant yielded one peak of bound radioactivity in or close to the void volume of the Sephadex G200 column. The elution position of the steroid–receptor complex was similar to that of receptors in epididymis and prostate supernatants[24,27], but different from the ^{3}H-DHT-ABP complex which has a Stokes radius of 47 Å (refs 6, 7, 24). The bound radioactivity was extracted and again identified by TLC and crystallisation as testosterone (55%) and DHT (45%). Centrifugation of these supernatants through 5–20% linear sucrose gradients (sucrose solution in 50 mM Tris HCl, *p*H 7.4, containing 1 mM EDTA, 0.5 mM 2-mercaptoethanol and 10% glycerol) gave one nondisplaceable peak of bound radioactivity sedimenting as 6–8S.

To establish the receptor identity of CR in testis supernatant, the temperature stability and the dissociation of the androgen–protein complex were compared with cytoplasmic receptor in epididymis. Twenty animals, 25-d-old, were hypophysectomised and injected with ^{3}H-testosterone 3 d later. Testicular and epididymal 105,000*g* supernatants were heated at either 25° C or 50° C for 30 min, and binding analysed by PAGE. Androgen–CR complexes in both testis and epididymis were stable after heating at 25° C, but were destroyed by heating at 50° C. Similar heat stability was found for the androgen–protein complex excluded from Sephadex G200 and the 6–8 S complex obtained by sucrose gradient centrifugation. This heat sensitivity was identical to that of the prostate cytoplasmic receptor[24,25,27], but different from that of ABP which was stable at 50° C (refs 6, 8, 24, 25). Dissociation of the labelled androgen–CR complexes was examined by PAGE after incubation of *in vivo* labelled supernatant with a large excess (10 μg) of unlabelled DHT for 2 h at 0° C. Dissociation of the androgen–CR complex in testis was negligible as was the dissociation of the androgen–receptor complex in epididymis. This extremely tight binding of androgen by the testis CR at 0° C ($t_{1/2}$ 0° C = 35 h) is similar to the binding by androgenic receptors in the epididymis and prostate, giving further support to a receptor function of testicular CR. Evidence for translocation of cytoplasmic androgen–receptor complexes into testis nuclei was obtained from the demonstration of androgen binding macromolecules in purified nuclei with steroid specificity identical to CR. These androgen–protein complexes could be extracted with 0.4–1 M KCl following injection of ^{3}H-testosterone and had a sedimentation of 3–4S.

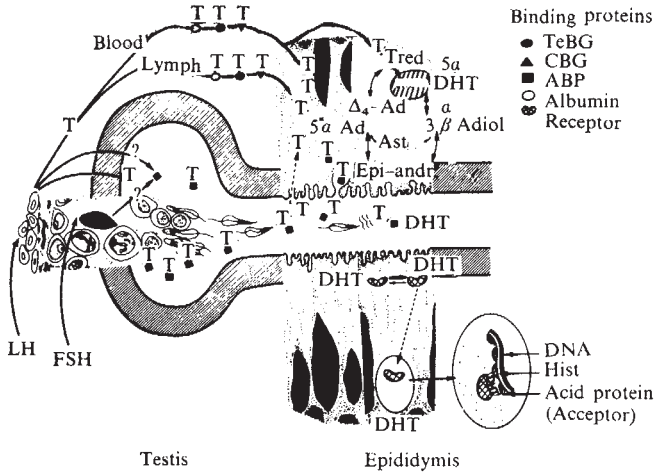


Fig. 5 Schematic drawing of androgen action in the testis and epididymis. Testosterone (T) is secreted from the Leydig cells due to LH stimulation. Most of the testosterone is removed from the testis bound to different binding proteins in blood and lymph. ABP is produced by the Sertoli cells due to FSH stimulation and secreted into the seminiferous tubules. There, ABP generates a diffusion potential causing a net inflow of testosterone and accumulation of androgens. FSH also causes an increased secretion of ABP (and androgen) from the testis into the epididymis, by the way of the efferent duct fluid. Testosterone which enters the epididymis is activated by the formation of DHT. DHT bound to receptors is translocated to the cell nucleus, initiating the different metabolic processes necessary for sperm maturation.
Δ_4 Ad = Δ_4-adione; 5α Ad = 5α adione;
Ast = Asterone; 3β Ad = 3β adiol; Epi-andr = epiandrosterone

The demonstration of androgen–receptor complexes in cytoplasmic and nuclear fractions of seminiferous tubules supports the concept that spermatogenesis is an androgen-dependent process. LH stimulates spermatogenesis by increasing androgen production by the Leydig cells. FSH stimulates production of ABP, presumably by the Sertoli cells, thereby increasing the binding and accumulation of androgen within the seminiferous tubules close to androgen-dependent cells of the epithelium (Fig. 5). ABP might, therefore, act as an essential androgen concentrating factor, which seems to be under control of FSH. In addition, FSH stimulation of ABP secretion into testicular fluid increases the amount of ABP (and androgen) transported to the caput epididymidis by the efferent ducts. Testosterone, which is taken up by epididymal cells both from the lumen and the circulating blood is 'activated' by 5α-reduction to form DHT. DHT bound to cytoplasmic receptors is transported into the cell nuclei where the androgen–receptor complex binds to chromatin and initiates metabolic processes which are necessary for sperm maturation. After releasing its bound testosterone to epithelial cells, ABP is free to bind testosterone or DHT which diffuses into the lumen from the blood or epithelium.

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Our results support the view that the germinal epithelium of rat testis is an androgen-dependent target tissue. The intracellular receptors for androgen demonstrated here probably mediate the androgenic stimulus to spermatogenesis. It is not yet clear which cells within the seminiferous tubules contain CR. Spermatocytes show typical 'target tissue' metabolism of ^{14}C-testosterone to DHT and 5α-androstane-3α,17β-diol[28]. Also the formation of these metabolites by the rabbit testis is greatly reduced after selective destruction of the germinal cells of the seminiferous tubules by heat[29]. The persistence of CR for as long as 24 d after hypophysectomy, however, indicates that CR is present in either Sertoli cells, spermatogonia or primary spermatocytes, since these were the only cell types remaining. To what extent the germinal epithelium cells are capable of retaining the CR–androgen complexes in their nuclei remains to be determined. Further studies are necessary to determine whether androgen acts directly on proliferating germ cells or stimulates spermatogenesis by an indirect action on the Sertoli cells.

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Nucleotide pyrophosphatase, a sialoglycoprotein located on the hepatocyte surface

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A sialoglycoprotein enzyme hydrolysing nucleotide pyrophosphate bonds is a major externally located component on the hepatocyte surface membrane.

MEMBRANE-BOUND enzymes play a paramount role in the control of cell metabolism. Enzymes located on the surface membrane occupy a crucial position for control of the interaction of cells with the external environment. To assess the role of cell surface membrane enzymes in metabolic regulation and cell surface phenomena, knowledge of the location of these enzymes, especially with regard to the outer or inner faces of the membrane is desirable. By using an enzymic technique[1] to label the surface membrane of isolated cells and a subcellular fraction derived from the liver cell surface, I show that nucleotide pyrophosphatase, an enzymic marker in plasma membrane isolation studies[2,3], is externally located on the hepatocyte.

Nucleotide pyrophosphatase was purified from liver plasma membrane as a sialoglycoprotein of molecular weight 130,000 and is a major constituent of liver plasma membranes[4]. The external location of the nucleotide pyrophosphatase accounts for the failure of nucleotides to enter the liver in metabolic experiments[5-7]. The fact that the nucleotide pyrophosphatase is on the outside of the cell, whereas the sugar transferases are on the Golgi apparatus[8,9], strongly suggests that mechanisms proposing a role for membrane transferases in intercellular adhesion[10,11], and the uptake of serum glycoproteins[12] are unlikely to be generally operative.

Iodination of liver plasma membranes

Plasma membranes were prepared from liver homogenates as a population of vesicular membrane profiles derived from the sinusoidal, canalicular and contiguous faces of the hepatocyte[2,13,14]. They were labelled with ^{125}I by using the lactoperoxidase-catalysed iodination procedure in which H_2O_2 was generated by a glucose-glucose oxidase system[1]. This iodination technique was shown to label specifically and covalently the external face only of erythrocytes, erythrocyte ghosts[1], liver 'microsomal' vesicles[15], and lymphocytes[16]. Iodinated, washed liver plasma membranes were dissolved in sodium dodecyl sulphate, and the polypeptides separated, mainly on a molecular weight basis, by electrophoresis in flat sheets of polyacrylamide[17]. Figure 1*a* shows liver plasma membranes to consist of at least 30 polypeptides, of which about six of the components in the higher molecular weight range are glycosylated. Iodinated plasma membranes contained two major peaks of ^{125}I-radioactivity corresponding to glycosylated polypeptide bands of apparent molecular weight of 130,000 and 100,000 (Fig. 1*b*).

The enzymic identity of one of the major iodinated polypeptides in liver plasma membranes was established by copurification of ^{125}I-radioactivity and nucleotide pyrophosphatase activity peaks. Since the enzyme also hydrolysed phosphodiester linkages[4], it was more convenient to monitor purification of the enzyme by measuring alkaline phosphodiesterase activity by hydrolysis of the artificial substrate thymidine 5′-monophosphate *p*-nitrophenol ester. Iodinated plasma membranes,

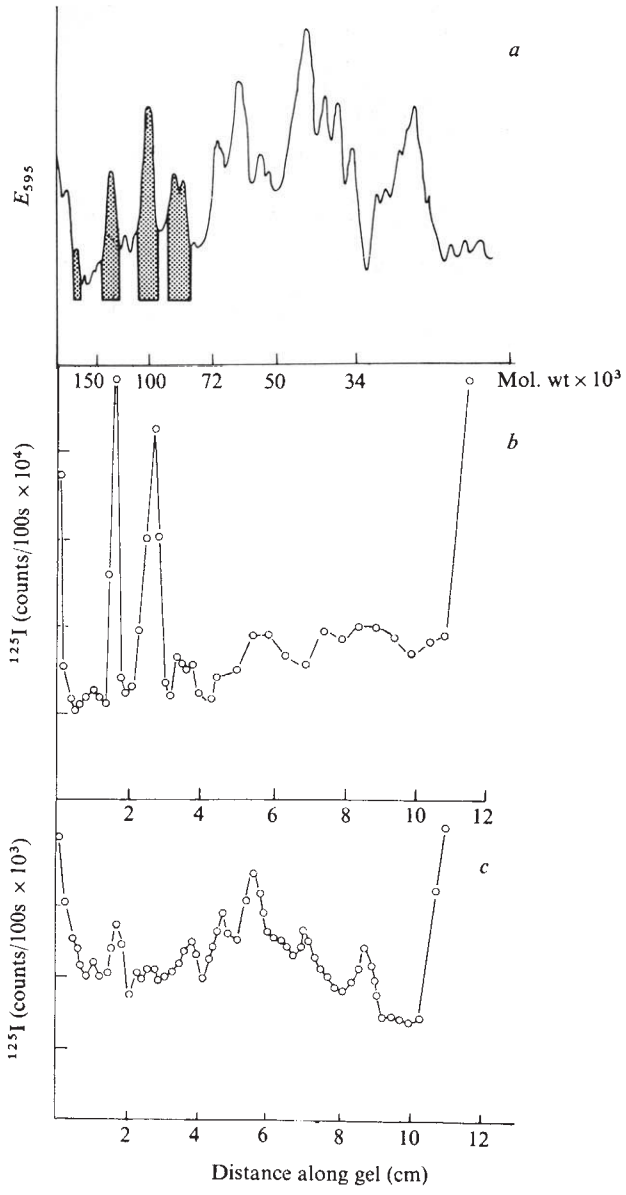


Fig. 1 Electrophoretic separation on polyacrylamide gels of iodinated liver plasma membranes (*a*, *b*) and isolated hepatocytes (*c*). Liver plasma membranes were prepared from 'nuclear' pellets by a rate zonal procedure[13], or from a 'microsomal' fraction by density gradient centrifugation[2,14]. Livers were dissociated by perfusion through the portal vein with hyaluronidase and collagenase[21]. Membranes (13 mg protein) or hepatocytes (1×10^7 cells) suspended in 0.8 ml phosphate buffered saline containing 5 mM glucose were iodinated with 0.4 to 0.6 mC; carrier-free ^{125}I for 15–30 min at 20°C in the presence of 100 μg lactoperoxidase (Sigma) and 24 μg glucose oxidase (Worthington Biochemicals)[1]. Iodinated membranes (*a*, *b*) were washed six times by centrifugation in water, and then dialysed against water until the dialysate was free of radioactivity, before dissolving by heating in 2% sodium dodecyl sulphate–4 M urea–1% mercaptoethanol. Iodinated hepatocytes (*c*) were washed six times in phosphate buffered saline by centrifugation at 50*g* for 2 min and then suspended for 15 min at 37° C in 1 ml phosphate-buffered saline containing 1% deoxycholate and 25 μg DNase. The supernatant, containing 90% of ^{125}I radioactivity attached to the cells, was mixed with sodium dodecyl sulphate–urea–mercaptoethanol. Membrane polypeptides and extracts of cells were resolved by flat plate electrophoresis in 8.5% polyacrylamide gels equilibriated in 0.1% sodium dodecyl sulphate[17]. Plasma membrane polypeptides were stained for protein with Coomassie Blue and the bands densitometrically traced at E_{595} (*a*). Glycoproteins (hatched areas) were identified by using the Schiff periodate reagent[18]. Gels were then cut into thin slices of equal length with a blade and ^{125}I-radioactivity determined on a γ-counter (*b*, *c*). In the absence of lactoperoxidase, fewer than 5% of the counts were incorporated and no peaks of radioactivity associated with protein were present in gel separation of membranes or cells.

solubilised in the detergent N-lauryl sarcosinate[18], were added to a Sepharose 6B column, and the resolution of ^{125}I-radioactivity, protein and two plasma membrane marker enzyme activities—phosphodiesterase and 5′-nucleotidase followed (Fig. 2). A close coincidence between a peak of radioactivity and of phosphodiesterase activity was observed. Column fractions corresponding to the phosphodiesterase and 5′-nucleotidase peaks were pooled, concentrated and analysed by polyacrylamide gel electrophoresis. Figure 3*a* indicates that the phosphodiesterase activity peak corresponds to the ^{125}I-radioactivity peak of molecular weight 130,000 present in plasma membranes (Fig. 1*a*, *b*). The 5′-nucleotidase activity peak from the column that overlapped the trailing edge of the phosphodiesterase activity peak (Fig. 2) also contained a small ^{125}I-radioactivity peak of molecular weight 130,000 but the main peak of ^{125}I-radioactivity was at molecular weight 100,000 (Fig. 3*c*).

A sialoglycoprotein with dual specificity towards nucleotide pyrophosphate and phosphodiester bonds was purified from liver plasma membrane as a single band on polyacrylamide gels of apparent molecular weight 130,000 (ref. 4). The above results, therefore, suggest that the enzyme was iodinated. But to examine more directly the relationship between the enzyme activities separated by gel filtration and peaks of radioactivity resolved on polyacrylamide gels, the two enzyme activity peaks collected from the Sepharose column were also electrophoresed in polyacrylamide gels equilibrated in buffers containing deoxycholate (Fig. 3*b* and *d*). Although the resolution of membrane components in gels equilibrated in deoxycholate-buffer was inferior to that obtained in sodium dodecyl sulphate-buffer as judged by protein staining, the major ^{125}I-radioactivity and phosphodiesterase peaks were coincident (Fig. 3*b*). But the major 5′-nucleotidase activity peak did not coincide with the ^{125}I-radioactivity peak of molecular weight 100,000 (Fig. 3*d*).

The overall results show that the alkaline phosphodiesterase-nucleotide pyrophosphatase of liver plasma membranes was iodinated by the adopted procedure, since enzyme activity, ^{125}I-radioactivity and the molecular weight determined independently on purified enzyme were coincident. The liver plasma membrane fraction prepared consisted of closed vesicles. Studies with similar vesicles of a liver microsomal fraction have shown that they are impermeable to lactoperoxidase, and only proteins on the external face of the microsomal membrane were iodinated[15]. One may conclude, therefore, that the sialoglycoprotein functioning as a phosphodiesterase-nucleotide pyrophosphatase is located on the outer surface of the liver plasma

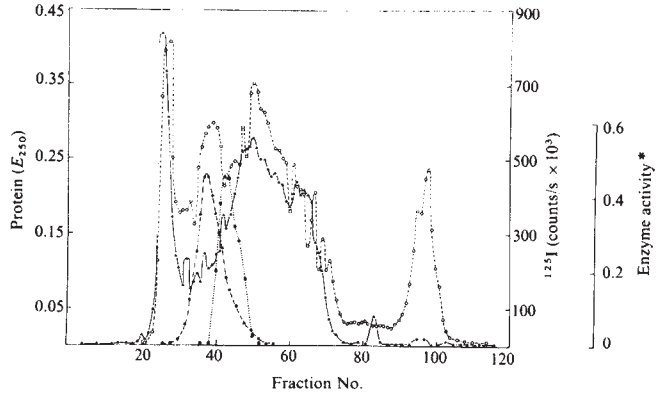


Fig. 2 Gel filtration on a Sepharose 6B column of a sarcosyl extract of iodinated liver plasma membranes. Iodinated membranes were extracted with a 4% sarcosyl, 2.1% Tris buffer *p*H 7.9[4,18], and the extract, after concentration by pressure filtration across Diaflow XM50 filters, was resolved in a column equilibrated with 0.2% sarcosyl buffer. Column fractions were assayed for protein at E_{280} (▲) ^{125}I (○), 5′-nucleotidase[7] (●) and alkaline phosphodiesterase[7] (■) activities. The activity peaks of phosphodiesterase (tubes 35–39) and 5′-nucleotidase (tubes 42–48) were concentrated by pressure filtration across Diaflow UM50 filters.
*Phosphodiesterase or 5′-nucleotidase (arbitrary units).

membrane vesicles. Carbohydrate chains of glycoproteins are externally orientated on surface membranes[19], and the liver plasma membrane contains sialic acid on its outer surface[20]. It is highly likely that the plasma membrane vesicles used in the present study were of a normal 'right side out' orientation, but to obtain further evidence for the external location on the liver surface membrane of the labelled sialoglycoprotein enzyme, free hepatocytes were prepared and iodinated in the same manner.

Iodination of isolated hepatocytes

Perfusion *in situ* of rat liver through the portal vein with a Krebs–Ringer medium containing collagenase and hyaluronidase dissociates the lobules into cells[21]. Hepatocytes, separated from the Kupffer, endothelial and other cells by repeated low-speed centrifugation, were iodinated as described for the plasma membrane fraction[1]. The iodinated cells were briefly incubated in a medium containing deoxycholate and the extracts then analysed by polyacrylamide gel electrophoresis in sodium dodecyl sulphate-buffer (Fig. 1*c*). Many peaks of ^{125}I-radioactivity were obtained, and one major peak corresponded in its molecular weight position to one of the two major iodinated peaks obtained with isolated plasma membranes. The coincidence in molecular weight of a ^{125}I-radioactivity peak of apparent molecular weight 130,000 both in a plasma membrane subfraction and cells supports the conclusion that the phosphodiesterase-nucleotide pyrophosphatase is located on the surface of the hepatocyte. The presence of a greater number of labelled components on hepatocytes compared with isolated plasma membranes contrasts with the results obtained with iodinated erythrocytes[1]. It may be caused by damage to the outer cell surface of the hepatocyte occurring during the enzymic treatment used to dissociate the tissue, or to the presence on the cells of tightly-adsorbed nonmembrane proteins.

Implications for membrane functioning

To resolve the molecular basis of events occurring at the cell surface, a number of chemical[22] and enzymically catalysed labelling reagents[1,23] have been used to identify molecules externally located on the surface membrane. These studies, initially made on erythrocytes[23] and later extended to include platelets[24], fibroblasts[25], lymphocytes[16,26] and cultured cells[27,28], in general identify glycoproteins of high molecular weight among the labelled components. For example, lymphocyte and sarcoma ascites tumour cell plasma membranes possess a major protein of molecular weight 130,000 that was labelled by iodination[26,29]. These studies do not, however, assign a direct functional role to the iodine-labelled cell surface membrane components. My experiments show that a glycoprotein component located on the outer face of the hepatocyte surface membrane possesses nucleotide pyrophosphatase activity.

The rapid degradation of nucleotides, for example, NAD, ATP, UDP-galactose by perfused livers[5] is now explained. Hydrolysis by the blood sinusoidal surface membrane of sugar nucleotides yields nucleotide monophosphates which may be further degraded by nucleotide monophosphatases, for example, 5′-nucleotidase which, although not significantly labelled in the present experiments, was also shown by the use of antisera[30] and by cytochemical studies[31] to be externally located on liver surface membranes. These enzymes may be components of a metabolic network of enzymes integrated to varying degrees into the surface membrane and functioning in the transport of nucleotides across the cell surface.

Cell surface galactosyl transferase activity transferring galactose from sugar nucleotides to galactosyl acceptors has featured in proposed intercellular contact mechanisms[10,11] and the enzyme was also implicated as a binding site on liver plasma membranes for asialoglycoproteins[12]. But subcellular fractionation studies of the liver have clearly distinguished between

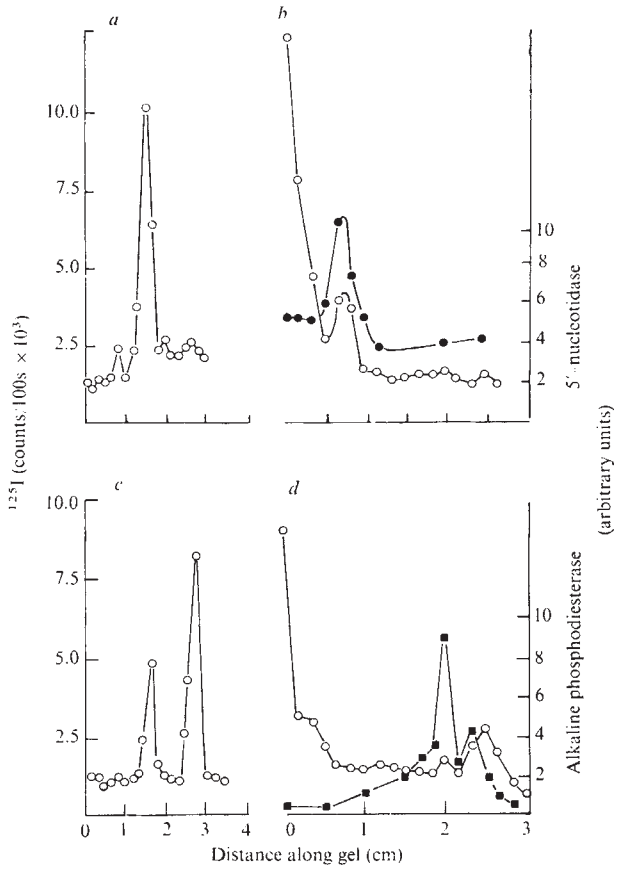


Fig. 3 Polyacrylamide gel electrophoresis of the alkaline phosphodiesterase (*a*, *b*) and 5′-nucleotidase (*c*, *d*) activity peaks separated by gel filtration (Fig. 2). The concentrated samples were electrophoresed in gels equilibrated in Tris-glycine buffers containing either sodium dodecyl sulphate (*a*, *c*) or deoxycholate (*b*, *d*)[4]. Enzyme activities (expressed as arbitrary units) were determined on dispersed gel fragments after determination of ^{125}I-radioactivity (○).

Golgi and plasma membrane fragments[8,9] and shown that galactosyl transferases are Golgi enzymes and are not significantly located on the plasma membrane. Therefore in liver, and probably other cells, galactosyl transferases are not directly involved in cell surface phenomena. My results reinforce this conclusion, since even if some transferases were on the cell surface, the presence of the nucleotide-phosphophatase would ensure a rapid disappearance of the transferase substrates, unless a lateral topographical separation of enzymic activities was invoked. Nucleotide pyrophosphatases–alkaline phosphodiesterases have been detected on a number of mammalian surface membranes[3,32,33] and such an enzyme is probably one of the surface components of approximate molecular weight 100,000 shown to be iodinated on various cells. But the generalisation that this enzyme is always located externally on the cell surface membrane as currently shown for hepatocytes, must await copurification of enzymic and radioactivities in other cells and tissues. Cultured hamster cell lines were shown indirectly to contain an externally located surface membrane nucleotide pyrophosphatase, and the enzymic activity was absent in virally transformed cells[31,34]. The present identification thus focuses on a molecular difference between normal and virally transformed cell lines of a cell surface sialoglycoprotein enzyme[35].

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Sequence of a repressor-binding site in the DNA of bacteriophage λ

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The sequence of 33 nucleotides immediately preceding the start point of transcription of an operon in bacteriophage lambda has been determined. This region includes recognition sites for lambda repressor and, probably, for other proteins. The sequence contains interdigitating symmetries.

Two loci on the DNA of bacteriophage λ—the operators—contain multiple repressor binding sites. Superimposed on each operator are sites recognised by a restriction endonuclease (Hin) and by *E. coli* RNA polymerase[1–5]. At each operator (Fig. 1), repressor preferentially binds to a terminal site and the remaining sites are filled in the direction opposite to that of transcription of the adjacent controlled operon. Operator O_L, for example, controls leftward transcription of gene *N*. Repressor (probably a dimer)[6] first binds to a 30–35 base pair site (S_1) proximal to *N*, and additional molecules (probably monomers) add sequentially to the right, filling five 15-base pair sites of lower repressor affinity. Each operator contains a single Hin target between the first and second repressor binding sites. At O_L, this site is located immediately to the right of S_1. The region of each operator recognised by RNA polymerase includes or is immediately adjacent to the Hin site[3,4]. O_L is not transcribed *in vivo* (ref. 7 and H. Lozeron, personal communication); the start-point of leftward transcription is to the left of S_1.

We report here the nucleotide sequence of 33 residues between the Hin site in O_L and the start point of leftward transcription. We also confirm the sequence of the first 26 residues to the left of the transcription start point, originally determined by analysis of an RNA transcript (J. E. Dahlberg and F. F. Blattner, in preparation). We refer to this latter sequence as the 5′ end of N, although it is not known where translation of N begins. We present here the essentials of our arguments and our conclusions; details of the sequence determination will be presented elsewhere.

Experiments and results

When λ DNA is digested with Hin, about 50 different double-stranded fragments are generated. One of these, which is about 1,125 bases long (Hin 1125) contains S_2–S_6, and another contains S_1 (Fig. 2)[2,3]. We purified microgram quantities of Hin 1125 by gel electrophoresis, and used it to prime *de novo* synthesis of one strand of S_1 as follows. Denatured Hin 1125 was reannealed in the presence of approximately equimolar quantities of purified λ *l* strand DNA. The *r* strand of the annealed Hin 1125 was then extended into S_1 by incubating the mixture with *E. coli* DNA polymerase I in the presence of Mn^{2+}, ribo-CTP or ribo-GTP and the appropriate three deoxynucleoside triphosphates,

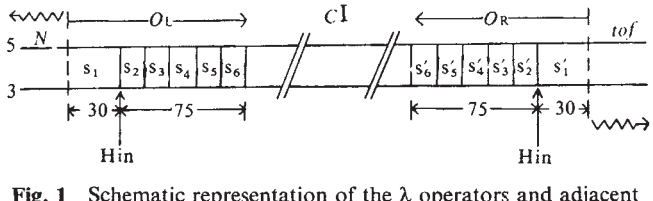


Fig. 1 Schematic representation of the λ operators and adjacent genes. The wavy arrows show the direction of transcription of the repressor-controlled genes *N* and *tof*. Two arrows indicate the order of repressor binding from the first sites (S_1 and S'_1) to the final sites (S_6 and S'_6) in O_L and O_R. The Hin cutting sites in O_L and O_R are indicated along with the number of base pairs within the operators on either side of each Hin cut. The repressor gene is *cI*.

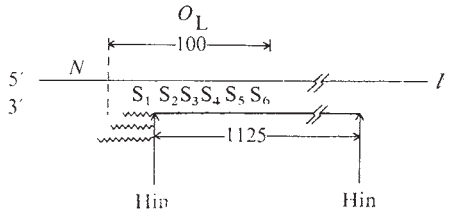


Fig. 2 Schematic representation of primed DNA synthesis at the leftward operator O_L. The lines labelled *l* and *r* represent, respectively, the *l* strand of λ DNA and the *r* strand of the restriction endonuclease fragment Hin 1125. Repressor binding sites within O_L are designated S_1–S_6. The boundary between O_L and the start-point of transcription of gene *N* is indicated by the dashed vertical line. The varying lengths of *in vitro* synthesised DNA resulting from asynchronous chain growth are represented by the wavy lines. The restriction endonuclease (Hin) cleavage sites at the extremities of Hin 1125 are indicated.

one of which was labelled in the α position with ^{32}P. The *in vitro* product of interest was then released from the template-primer complex by Hin digestion followed by denaturation. In some cases the product was digested with RNase without Hin digestion. The labelled oligonucleotides were fractionated and

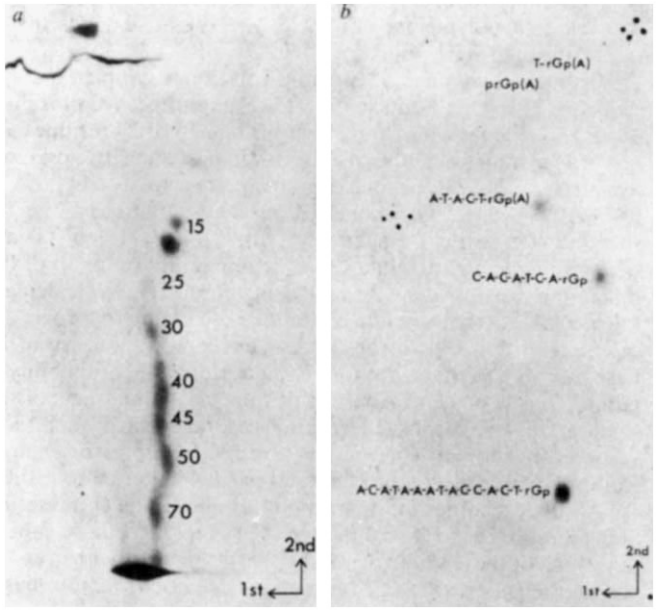


Fig. 3 *a*, Two-dimensional fractionation of the products of a restriction-fragment-primed DNA polymerase I reaction. A total volume of 250 μl contained: 12 μg purified λ *l* strand DNA[18], 1 μg Hin 1125 DNA[2], 67 mM Tris-HCl, *p*H 7.4, 0.67 mM $MnCl_2$, 1 mM 2-mercaptoethanol, 1 mM ribo-GTP, 0.025 mCi α-^{32}P-dATP (100 Ci mmol^{-1}), 0.05 mM dTTP, 0.05 mM dCTP, 20 μl DNA polymerase I (approximately 1–5 units). The reaction was started by the addition of DNA polymerase and incubated at 8° C. After 30 min the reaction mixture was extracted with phenol, and residual phenol removed by several ether extractions. The products were dialysed overnight against Hin buffer[2,3]. To release the newly synthesised DNA from the restriction fragment, the sample was digested for 2 h with exonuclease-free Hin prepared as described previously[3]. The sample was precipitated by the addition of 1/10 volume 3 M sodium acetate and two volumes of ethanol. The precipitate was taken up in 5 μl of H_2O, sealed in a capillary, heated to 100° C and cooled in an ice bath. The denatured sample was fractionated in the first dimension by electrophoresis on cellulose acetate in a *p*H 3.5 buffer containing 5 mM EDTA and 7 M urea. The second dimension was homochromatography using a 7% unhydrolysed RNA mixture. The approximate chain lengths of the products are shown. *b*, Two-dimensional fractionation of an RNase digest of a ribo-G-substituted oligonucleotide. The sequences of the various products are indicated on the figure. The primary product, about 40 bases long, was eluted from the homochromatography plate of (*a*) and digested overnight with 5 μl of a solution containing 10 mg ml^{-1} RNase T_1 in 0.05 M Tris-HCl, *p*H 7.4, and 0.005M EDTA. The digest was fractionated as in (*a*) except that a 3% RNA mixture hydrolysed for 30 min was used in the second dimension.

their sequence was determined using methods described by Sanger *et al.*[8].

A typical experiment was as follows. The product of a Hin 1125-primed synthesis, polymerised from rGTP, dTTP, dCTP and ^{32}P-labelled dATP, was separated from the template primer complex by Hin digestion and subjected to two dimensional fractionation (Fig. 3). The homochromatography mixture used in the second dimension separates oligonucleotides of lengths about 1–80 bases. The products seen in Fig. 3*a* have a common 5′ end (produced by Hin digestion) but differ in the 3′ ends because of asynchronous growth of the chains. Each fractionated product was eluted, digested with ribonuclease T_1, and again subjected to two-dimensional fractionation. Figure 3*b* shows a fingerprint of the oligonucleotides generated by RNase T_1

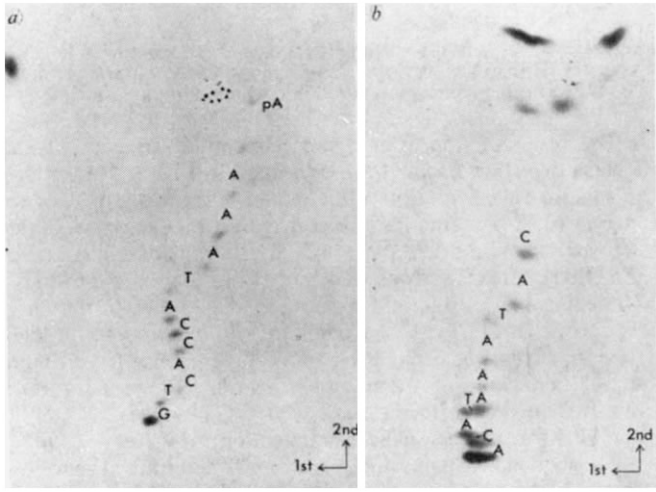


Fig. 4 Two-dimensional fractionations of snake venom exonuclease (*a*) and spleen exonuclease digests (*b*) of the 15-base long ribo-G terminated product, A-C-A-T-A-A-A-T-A-C-C-A-C-T-rGp. The digests were fractionated as described in the legend to Fig. 3. From the distance and angle of shift between a digestion product and that product with one base removed the identity of the removed base can be determined[8]. The nucleotide removed is shown between each product. Thus the venom exonuclease digestion gives a 3′ sequence of A-A-A-T-A-C-C-A-C-T-G and the spleen exonuclease gives a 5′ sequence of A-C-A-T-A-A-A-T-A-C. These two sequences contain the common sequence A-A-A-T-A-C. Digestion conditions were as follows. For venom exonuclease the sample was incubated first with 3 μl of a solution containing 10 mg ml^{-1} bacterial alkaline phosphatase, 0.05 M Tris-HCl, *p*H 8.9, and 0.005 M $MgCl_2$ for 30 min at 37° C, to remove 3′ phosphate. 4 μl of a solution containing 0.1 mg ml^{-1} snake venom phosphodiesterase, 0.05 M Tris-HCl, *p*H 8.9, 0.05 M $MgCl_2$ was then added and the sample incubated at 37° C, and 1 μl aliquots were taken at 0, 15, 30, 45, 60, 90, and 120 min, frozen, and then pooled. For spleen exonuclease digestion was with 7 μl of a solution containing 0.1 mg ml^{-1} spleen phosphodiesterase, 0.05 M citrate buffer *p*H 6.0. The reaction was treated as in (*a*).

digestion of one of these products, about 40 residues long. In this case the homochromatography mix used in the second dimension separates oligonucleotides of lengths about 1–20 bases. By comparing the fingerprints generated in this way from each of the Hin digestion products seen in Fig. 3*a*, the order of synthesis of various ribo-G-containing oligonucleotides, labelled with ^{32}P-dATP was determined. Finally, the sequence of the various ribonuclease T_1 products was determined by partial exonuclease digestion and by nearest neighbour analysis. Figure 4*a* shows a two-dimensional fractionation of a partial venom phosphodiesterase digest of an RNase T_1 product identified in the experiment of Fig. 3*b*. Figure 4*b* shows a partial spleen phosphodiesterase digest of the same product. (Venom and spleen phosphodiesterase attack, respectively, from the 3′ and 5′ ends of polynucleotide chains.) Inspection of these fingerprints reveals the following sequence, which was confirmed by nearest neighbour analysis (not shown):

5′ G/ACATAAATACCACTG/G. 3′

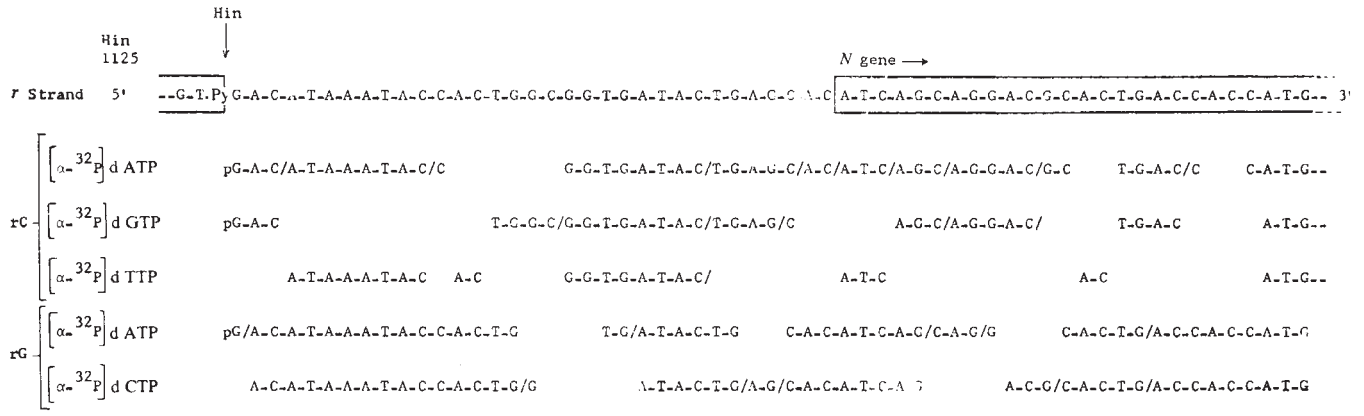


Fig. 5 The sequence from the Hin site of O_L to residue 26 beyond the startpoint of transcription of *N*. The sequences and order of synthesis from the Hin site of various ribo-C and ribo-G containing oligonucleotides are shown. The ribonucleotide triphosphate and the labelled deoxynucleotide triphosphate used in each reaction are indicated on the figure. Note that the orientation of the sequence is the reverse of that in Figs 2 and 6. See text for explanation.

The order of appearance and nucleotide sequence of each RNase digestion product are presented in Fig. 5. Note that in this figure the orientation of the sequence is the opposite of that in Fig. 6. This maintains the convention that oligonucleotides are written in the orientation 5′–3′. The complete sequence from the Hin site in O_L to *N* as well as that of the first 26 residues of *N*, deduced from this information, is also shown in the figure. The sequence GT Py (Py = pyrimidine, Pu = purine) to the left of the Hin cut in Fig. 5 has been added by inference from the fact that Hin II, one of the activities in our Hin preparation, is reported to recognise the sequence 5′ GTPyPuAC 3′ (ref. 10, and K. Murray, personal communication).

The sequence of a mutant operator was determined as follows. Experiments were performed using as primer wild type Hin 1125 and as template *l* strands isolated from λ*v101*. This phage bears a mutation that decreases the affinity of S_1 for repressor[2,3]. We found a single base difference between the wild type and λ*v101* sequences. The sequence ATAAATAC in wild type DNA is replaced in λ*v101* DNA with ATAAATGC.

Interesting aspects of sequence

The complete double stranded sequence of the region between the O_L Hin site and the beginning of *N* is shown in Fig. 6. Because RNA polymerase binds to the Hin site[3–5], the maximum number of base pairs separating bound polymerase from the transcription start point is 33. This value is consistent with our previous estimate[3] and is considerably less than that suggested by Blattner *et al*[10]. A revised estimate from Blattner *et al.* (personal communication) agrees with our data. DNA-bound polymerase is reported to cover 40 base pairs[11], and so it is possible that polymerase bound to the Hin site extends to the transcriptional start point.

There are three axes of two-fold rotational symmetry in the sequence in Fig. 6: at nucleotide 17 (Fig. 6*a*), between residues 15 and 16 (Fig. 6*b*), and at residue 14. In *a* 16 of 24 residues are perfectly symmetric and 6 of the remaining 8 show pyrimidine–pyrimidine—or purine–purine—symmetry. In *b*, 14 of 26 residues are perfectly symmetrical and 6 of the remaining 12 show Py–Py or Pu–Pu symmetry. In *c*, 14 (or 16) of 30 are perfectly symmetrical while 12 of the remaining 16 (or 10 of the remaining 14) show Py–Py or Pu–Pu symmetry. We calculate the probability that a region containing the symmetry properties of configuration *a* or *c* appears by chance in a sequence of 35 base pairs is less than 1%; for configuration *b* the probability is perhaps a few percent. Analysis of the pyrimidine tracts of the smallest fragment protected from nuclease digestion by repressor[1] suggests that the minimal region covered by repressor extends from about five residues to the right of the Hin target to residue 30. All three symmetries lie within this region. The O^c mutation at position 10 lies in two of the symmetry regions, but we cannot say which of these symmetries (if either) is recognised by repressor. In the *lac* operator O^c mutations have been found in bases

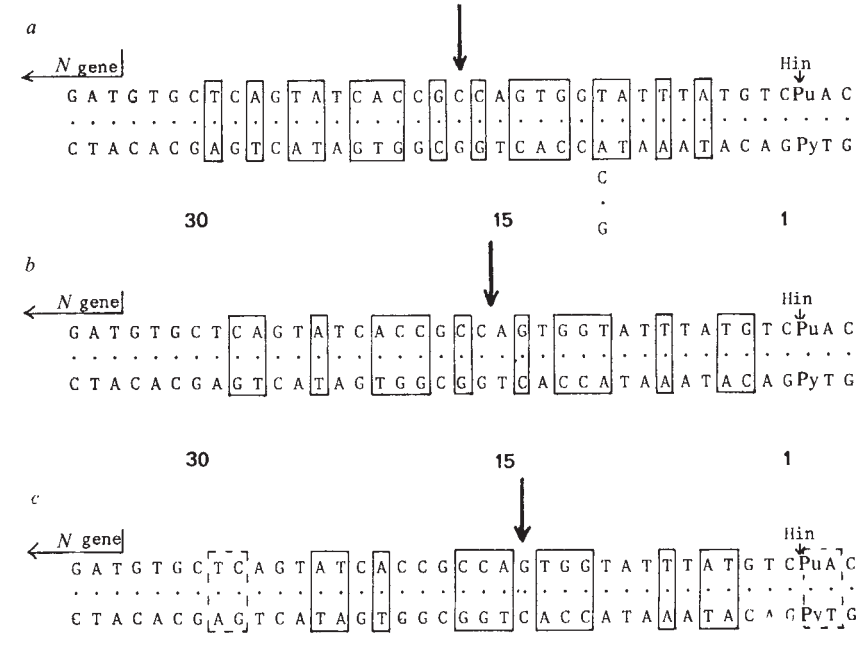


Fig. 6 Symmetries in the region between the Hin site in O_L and gene *N*. The bases in boxes are symmetric about two-fold rotational axes indicated by vertical arrows. An operator mutation at position 10 is indicated.

comprising a symmetric sequence, as well as in interspersed bases which do not contribute to the symmetry[12–14].

Why (other than fortuitously) should there be three overlapping (interdigitating) symmetries between the O_L H in site and N? One intriguing possibility is that these symmetries are recognised by different regulatory proteins—'multiplexing', as R. Pollack calls it. For example, the *tof* gene product is believed to act at O_L because a mutation which reduces the affinity of O_L for repressor also decreases the effect of *tof* product on leftward transcription[15]. RNA polymerase and N-product are two other proteins which might recognise these symmetries[16,17]. We note that it is much more difficult, if not impossible, to construct interdigitating symmetries using perfect as opposed to hyphenated symmetries such as those seen in Fig. 6. Sequence analysis of the remaining sites in the operators and localisation of various regulatory mutations should provide a clearer understanding of the significance of these symmetries.

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letters to nature

High velocities and cocoon stars

LARGE scale gas movements with velocities of the order of 50 km s^{-1} giving rise to split, asymmetric or broadened emission line profiles have been observed in many H II regions[1–4]. Proposed explanations for these motions include supernova explosions within or near to the H II regions[5], and the acceleration of their ionised material by relativistic particles[6] or by massive stellar winds[7–9]. I suggest that the breakup of the neutral gas and dust shell around a cocoon star could conceivably also produce high velocities. The existence of cocoon stars seems fairly well established from observations of infrared point sources[10,11] and these are frequently associated with compact H II regions[12] and OH masers. Kahn[13] investigated the evolution of a cocoon star and found that a star which accretes matter will form a false photosphere with a radius of the order 10^{13} cm and no appreciable H II region, provided that the rate of accretion exceeds approximately 10^{20} g s^{-1}. Around the false photosphere is a dust-free region with a radius of about 10^{16} cm, which corresponds to the radius at which refractory particles melt. At accretion rates of less than 10^{22} g s^{-1} the radiation pressure on the dust becomes powerful enough to stop the infall of matter and to drive a shock wave into the accreting gas flow outside a radius of 10^{16} cm. This situation is Rayleigh–Taylor unstable, and the shell will break up into globules. Consider a portion of neutral material with a density of M g cm^{-2}, at a distance, R, from a star with mass, M_*, and luminosity L_* ($M_* \approx 6 \times 10^{34}$ g, $L_* \approx 6 \times 10^{38}$ erg s^{-1}). If the radiation force just balances the gravitational force exerted by the star, then

$$M = L_* (4\pi c G M_*)^{-1} \quad (1)$$

Thus, $M \approx 0.7$ g cm^{-2} independent of distance. This is very close to the surface density required by the models of Dyson[14] ($M \simeq 2$ g cm^{-2}) for which a great deal of observational evidence[15,16] has been collected, particularly in the case of M8 and M42. It is thus possible that some of the globules represent the material that was just balanced by the radiation—gravitational forces, at the time of the breakup of the cocoon.

Next, consider the period of the onset of instability, when the accretion rate drops below 10^{22} g s^{-1}. The false photosphere will break up in certain directions according to a time scale given approximately by the free-fall time from a radius, R ($\simeq 10^{16}$ cm) to the star. That is about 10^{10} s. Thus, an H II region is formed very soon after the onset of the instability which quickly spreads through the dust-free cavity around the star. An estimate of the properties of the H II region can be obtained.

The density distribution of a freely falling gas cloud in steady flow is given by

$$\rho(R) = \dot{M} (32\pi^2 G M^*)^{-1/2} R^{-3/2} \quad (2)$$

where $\dot{M}$ is the accretion rate. Putting $\dot{M} = 10^{22}$ g s^{-1} and integrating out to $R = 10^{16}$ cm, the density in the initially formed H II region will be of the order of 10^8 cm^{-3} and the total mass involved will be of the order of 2×10^{31} g. This 'ultra-compact' H II region could not be contained by the overlying neutral material, and so would punch a hole (or holes) through those parts of the cloud with the lowest density, finally allowing the ionised material to escape more or less unimpeded.

The expansion of the ionised gas can be regarded as virtually isothermal, and so the ratio of the velocity, V, attained by a mass element and the sound speed, V_s, in the gas will be approximately given by:

$$V/V_s = (2 \ln(\rho_i/\rho_f)/3)^{1/2} \quad (3)$$

Where ρ_i is of the order 10^8 cm^{-3} and ρ_f is the final density, which is put equal to the density of a typical H II region—about 10^3 cm^{-3}. Thus, velocities of the order of three times the speed of sound should be attained along rather directional

streams. This is sufficient to explain the high velocities observed in a small fraction of the nebular material.

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Long term periodicities in the sunspot cycle

The existence of a long period undulation (~179 yr) in the solar cycle (Fig. 1, based on data from Waldmeier[1,2] is suggested by the planetary theory of sunspots. Jose[3], for example, postulated an association between the 178.8-yr periodicity in orbital positions of the planets and the phase of the solar cycle. Wood[4] used the presumed existence of a cycle of 170–180 yr in solar activity to predict the peak dates of future sunspot cycles. Our work indicates that although a 179-yr periodicity does exist in the sunspot cycle, it is not caused by a primary long term excitation function but is, instead, a beat phenomenon. This can be seen by computing the spectrum for the data shown in Fig. 1.

Burg (unpublished; see also ref. 5) suggested a method for estimating the spectrum of a time series that is based on an autoregressive description of the data to be analysed. This method, 'maximum entropy spectral analysis' (MESA), yields a result consistent with the information of the data and nothing more. We used this method in our initial analyses of the sunspot cycle.

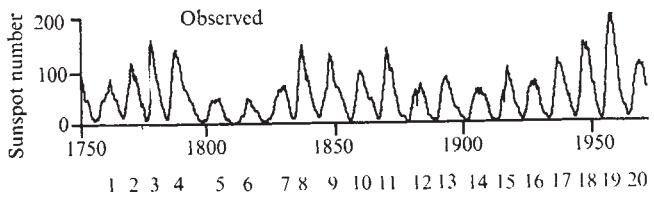


Fig. 1 Twelve-month running mean sunspot numbers (1750–1971), based on data given by Waldmeier[1,2]

Because the quality of early sunspot data is considered suspect by Waldmeier[1], we first analysed only data acquired after 1800. Specifically, for reasons related to computational procedures, we computed the spectrum for the 12-month running mean sunspot numbers from 1844 to 1971. The

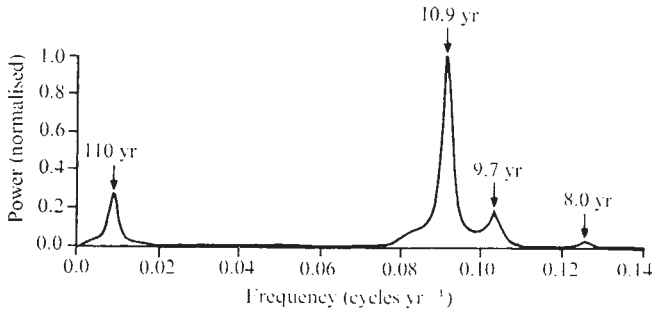


Fig. 2 Maximum entropy spectrum for the data interval 1844–1971.

MESA spectrum (Fig. 2) suggests that no significant power is present at periods greater than about 110 yr. The spectrum is dominated by three peaks with periods of about 110, 10.9 and 9.7 yr. Further, we note that the spectral components with periods 10.9 yr and 9.7 yr are separated in frequency by about 0.012 cycle yr^{-1}, and so the two sinusoids interfere with one another to produce beats with a period of roughly 167 yr. The similarity in behaviour of cycles 1, 2 and 3, and of cycles 17, 18 and 19 (Fig. 1) is apparently caused by this beat phenomenon.

That a long term periodicity can be observed in the data lends credence to the sunspot observations between 1750 and 1850. Therefore, we computed the MESA spectrum for a set of data which included the early mean sunspot observations. That analysis, which used 214 yr of data (1750–1963), yielded a spectrum with significant

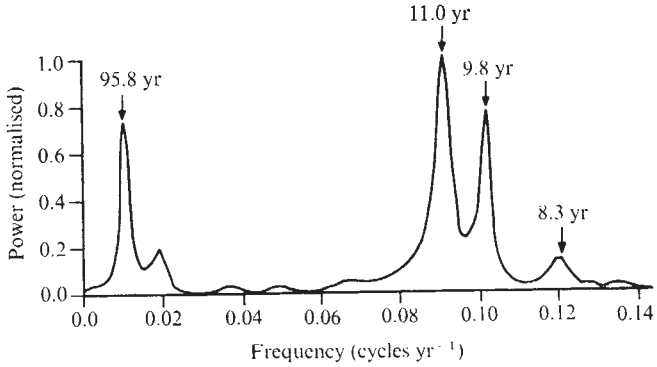


Fig. 3 Maximum entropy spectrum for the data interval 1750–1963.

signatures at periods of 95.8, 11.0 and 9.8 yr (Fig. 3). The frequency separation for the 11.0 and 9.8 yr spectral components yields an estimate of 181 yr for the long term periodicity. The similarity between the MESA spectra for the more recent sunspot observations (1844–1971) and the data incorporating the early observations (1750–1963) suggests that the sunspot data from 1850 to the present probably belong to the same population. This observation differs from the results of McNish and Lincoln[6], who hypothesised that the very early data and the more modern data belong to two statistically different populations.

To refine our determination of the frequency separation between the 11.0 and 9.8-yr spectral components, we used all of the sunspot observations shown in Fig. 1 and correlated these data with a suite of sinusoids finely spaced in frequency about the peak frequencies in the 214-yr maximum entropy spectrum. The best correlations occurred at frequencies which correspond to periods of 11.2 and 9.9 yr. The actual frequency determinations for these correlation peaks are separated by 0.01116 cycle % yr^{-1}, and as such, using this method the estimated period for the long

term undulation is 179 yr. This determination is in agreement with the average elapsed time between the peaks of cycles 1 and 17, 2 and 18, 3 and 19, and 4 and 20. Thus, although we estimate the length of the long period undulation in the sunspot cycle to be between 179 and 181 yr, we favour the 179-yr determination. This is somewhat longer than the 160–170 yr periodicity that Schove[7] observed in his data. On the other hand, our estimate is roughly 11 yr shorter than that of Cole[8], who observed a 190-yr phase variation in Schove's data. Regardless of these differences, the existence of a long period (160–190 yr) variation in sunspot activity seems certain.

If the major characteristics of the sunspot cycle repeat every 179 yr then, by definition, the data are periodic outside an interval of this length. It should, therefore, be possible to compute a periodogram using data covering 179 yr which is very similar to maximum entropy spectra computed previously. For example, the spectrum for the interval 1793–1971 (Fig. 4), exhibits the same spectral characteristics found in maximum entropy spectra computer previously.

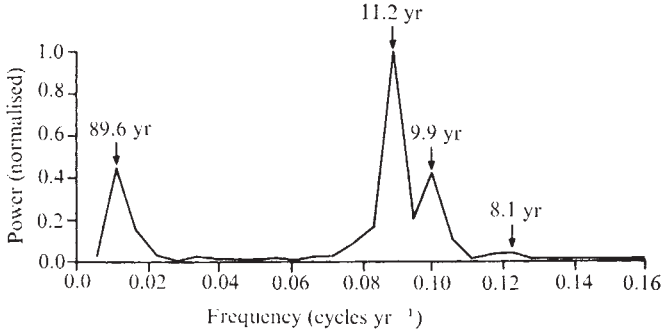


Fig. 4 Periodogram for the data interval 1793–1971.

We used the observation of the 179-yr periodicity to predict future sunspot activity. The ability to predict solar activity is important if efficient use is to be made of the high frequency spectrum for international communications. King[9] has shown, however, that significant climatic features (such as droughts and unusually long growing seasons) may be related to the solar cycle; if such relationships can be established with certainty, long range weather forecasts can then be issued for agricultural planning purposes (see also ref. 10).

Sunspot activity could be predicted by taking the spectral components corresponding to the data for any 179-yr interval and then reconstructing the smoothed sunspot numbers in that interval. Then, by repeating the waveform outside the interval, smoothed sunspot numbers could be extrapolated forwards and backwards in time. This method was applied to the spectral components corresponding to the periodogram of Fig. 4 (1793–1971) together with a component of 47.4 smoothed sunspots (components at periods of less than 4.5 yr were eliminated). That yielded the results shown in Fig. 5*A*. In the interval 1793–1971, the reconstructed waveform has an r.m.s. residual (Fig. 5*B*) of five 12 month running mean sunspots.

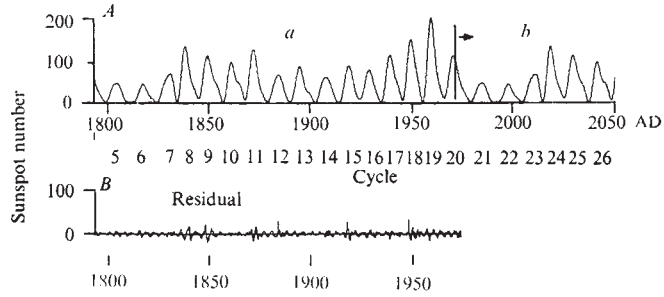


Fig. 5 Twelve-month mean sunspot numbers. *A*: *a*, reconstructed (1793–1971); *b*, predicted (1972–2050). *B*, Predicted minus reconstructed (1793–1971).

Because these extrapolations can be projected backwards as well as forwards in time (we have assumed that the data are periodic, with a period of 179 yr), it follows that the predictions for the interval 1750–1792 correspond to the estimated sunspot numbers in the interval 1929–1971. As a measure of our prediction capability, therefore, we compared our predicted mean sunspot numbers for major features of the sunspot cycle from 1750 to 1792 inclusive with the corresponding observed values (Fig. 6). Although the data suggest that our predictions for the smoothed numbers at sunspot maxima may be in error by up to ± 25%, and that the time estimates for the occurrence of sunspot minima and maxima may differ from the true times by up to ± 2 yr, the general agreement between the trends of the observed and the predicted sunspot numbers is considered to be good.

Using the data in Fig. 5*A*, several predictions can be made:

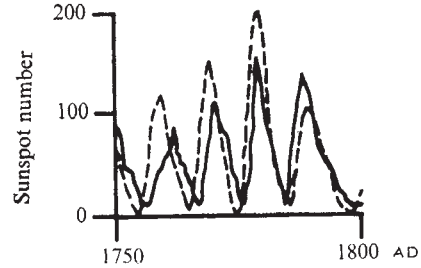


Fig. 6 Twelve-month running mean sunspot numbers (1750–1800). Solid curve, observed; dashed curve, predicted (1929–1971).

The current cycle, number 20, will exhibit a prolonged decay in sunspot activity, with a null-to-null period of nearly 13 yr. The activity of cycle 20 has already exhibited unusual decay behaviour by rising briefly, instead of falling continuously, in 1972. It seems plausible, therefore, that the next sunspot low will not be reached until after 1975, and possibly as late as the summer of 1977. At the minimum, mean sunspot numbers could be as low as 3.

Cycle 21 will have a rather broad peak, possibly reaching its maximum value late in 1982. Mean sunspot numbers for this peak may not, however, exceed 50. The sunspot minimum following cycle 21 should occur around 1988, and could exhibit smoothed sunspot numbers as low as 2.

Twelve month running mean sunspot numbers greater than 100 will not be observed again until approximately 2015. Thus, the next 40 yr may be characterised by relatively low sunspot activity as compared with the activity of the last 40 yr. This observation is supported by the results of Cole[8].

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Asymptotic structure in torsional free oscillation data for the Earth

THE eigenfrequencies, ${}_n\sigma_l$, of the torsional modes, ${}_nT_l$, of a spherically symmetrical, nonrotating, elastic, isotropic Earth model, are defined, for fixed wave number $k=(l+\frac{1}{2})/a$, by the eigenvalue problem

$$\mu\left[\frac{d^2U}{dr^2}+\frac{2}{r}\frac{dU}{dr}\right]+\frac{d\mu}{dr}\left[\frac{dU}{dr}-\frac{U}{r}\right]+\left[{}_n\sigma_l^2\rho-\frac{l(l+1)}{r^2}\mu\right]U=0$$

(where $U=U(r)$; $\mu=\mu(r)$; $\rho=\rho(r)$; $a\leqslant r\leqslant b$)

and by the (zero stress) boundary conditions

$$\mu=[dU/dr-U/r]=0,\ r=a,\ r=b;$$

where μ denotes rigidity, ρ density, a the radius of the core and b the radius of the Earth[1].

Using the Liouville transformation,

$$U=r^{-1}(\rho\mu)^{-1/4}Z,\ r=\int_a^s \mu^{1/2}\rho^{-1/2}\,dt+a,$$

the asymptotic behaviour of ${}_n\sigma_l$ can be expressed in the form

$${}_n\sigma^2{}_l=(n\pi)^2\gamma^{-2}+A\gamma^{-2}+B\gamma^{-2}n^{-2}+0(n^{-3})$$

for fixed l and suitably large n, where $\gamma=\int_a^b \rho^{1/2}\mu^{-1/2}\,dr$, and A and B are constants which depend only on the shear velocity and the density in the mantle, and on the radius of the core.

By its definition, γ corresponds to the radial travel time of an S wave between the surface of the Earth and the core–mantle boundary. On the basis of the asymptotic formula given, γ can be approximated by:

$${}_n\gamma_l^{(m)}=\pi(2nm+m^2)^{1/2}({}_{n+m}\sigma_l{}^2-{}_n\sigma_l{}^2)^{-1/2}$$

for sufficiently small values of m, if second order effects in the asymptotic formula are negligible. The values of ${}_n\gamma_l^{(m)}$ obtained from this expression may thus be expected to reach an asymptote, which is γ, for a sufficiently large overtone number $n=n_0$. Though n_0 is a function of l, it will be approximately constant over a small range of l values. This has been verified extensively using model data. For a given l, we estimate n_0 as the overtone number n at which, and beyond which, the values of ${}_n\gamma_{l-k_1}{}^{(m)}$, ${}_n\gamma_l{}^{(m)}$ and ${}_n\gamma_{l+k_2}{}^{(m)}$ match sufficiently closely. For example, Fig. 1 shows values of ${}_n\gamma_l{}^{(m)}$ calculated from the Earth model HB1 of Haddon and Bullen for $m=1$ and $l=2$, 18. The slight oscillations of the two curves about the asymptote in Fig. 1 indicate that second order

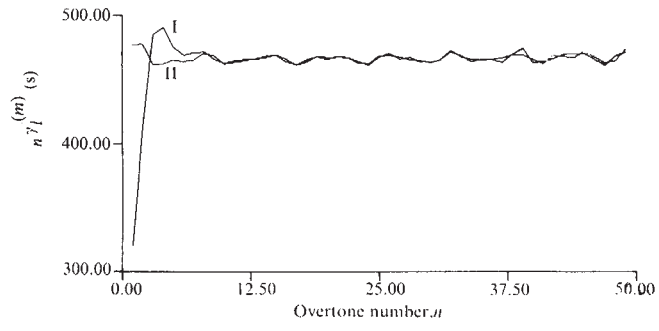


Fig. 1 Curves of ${}_n\gamma_l{}^1$ calculated from the Haddon and Bullen Earth model, HB1 (ref. 2): I, $l=2$, $n=1$ to 49; II, $l=18$, $n=1$ to 49.

effects are not completely negligible in the asymptotic formula which applies to torsionals computed from the Earth model HB1 (ref. 2). The structure in those second order effects is influenced by the position, size and sharpness of discontinuities in shear velocity, and by density in the mantle. The slight variation between the two curves for $n\geqslant 10$ is mainly because of numerical error in the computed periods.

The quantities γ and n_0 are obviously potentially important as constraints in the derivation of realistic Earth models by the inversion of torsional overtone data. Published observations of ${}_nT_l$ from free oscillation studies exist only for small overtone numbers[2], and calculations indicate that the asymptote has not been reached for these values of n. Some periods for ${}_nT_l$ up to $n=18$ have, however, been derived[3] from data[4] based on observations of the phase spectra of multiply-reflected S_0S waves. In particular, Brune and Gilbert[3] list periods calculated for ${}_8T_{14}$, ${}_{12}T_{14}$, ${}_9T_{16}$, ${}_{14}T_{16}$, ${}_{10}T_{18}$, ${}_{16}T_{18}$, ${}_{11}T_{20}$ and ${}_{17}T_{20}$, with a standard error of approximately 1.2 s. Estimates of γ derived from these observations are listed in Table 1. The range of these four estimates is 1.22 s, and for the last three it is only 0.38 s. Despite the smallness of the sample, the consistency of these results strongly suggests that $n_0<10$ for $14\leqslant l\leqslant 20$.

We have re-examined the data of Brune[4] to see whether there is any additional information about the overtone structure. Included in example 4 of his Table 1 is a period of

Table 1 Values of ${}_n\gamma_l{}^{(m)}$ derived from the data of Brune and Gilbert

n	l	m	${}_nT_l$ periods (s)	${}_{n+m}T_l$ periods (s)	${}_n\gamma_l{}^{(m)}$ (s)
8	14	4	109.64	75.81	469.30
9	16	5	97.18	65.10	470.14
10	18	6	87.29	75.03	470.44
11	20	6	79.27	53.54	470.52

147.659 s, which was not identified by Brune and Gilbert[3], but which must correspond either to ${}_6T_{10}$ or ${}_6T_{11}$. Using Brune and Gilbert's[3] periods for ${}_9T_{10}$ and ${}_{10}T_{11}$, this gives either ${}_6\gamma_{10}{}^{(3)}=466.91$ s or ${}_6\gamma_{11}{}^{(4)}=464.18$ s. Both are significantly smaller than the values of ${}_n\gamma_l{}^{(m)}$ in our Table 1, suggesting that $n_0>6$.

Brune and Gilbert acknowledge the possibility of bias towards continental values in the data, but point to the consistency with other normal mode data as an indication that there is no important regional bias. Although the value 470.36 s for the radial S time between the Earth's surface and the core–mantle boundary, obtained as the mean of the last three values in Table 1, does depend heavily on the 'structure' corrections applied by Brune[4] to his data, the accuracy of the numerical calculation is unaffected. As far as the estimation of n_0 is concerned, we have found from model studies that the point at which the asymptote becomes discernible depends largely on the position, size and sharpness of discontinuities in the upper mantle, and that smoothing of the discontinuities tends to shift this point to smaller values of n. The periods of free oscillation modes are determined by the laterally averaged properties of the Earth, and it is certain that some smoothing of the velocity and density structure in the region of the discontinuities would result from this averaging process. Brune (personal communication) has pointed out that the Brune and Gilbert[3] ${}_nT_l$ periods are strongly biased towards a model with no discontinuities, as a result of the fact that no reflections from any possible discontinuities were taken into account in the phase correlation. This no doubt accounts for the small scatter observed in the ${}_n\gamma_l{}^{(m)}$ values derived from these regional data. Our estimate of n_0 may therefore have to be revised when better data become available.

Finally, we remark that the nature of the asymptotic structure in the ${}_nT_l$ periods will impose a quite delicate constraint on Earth models, especially in view of the effects of upper

mantle discontinuities noted above. An investigation of the significance of this constraint is now in progress.

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H_2SO_4-HNO_3-H_2O ternary system in the stratosphere

RECENTLY, Friend *et al.*[1] reported a comprehensive laboratory study of a system of air containing trace quantities of H_2O, SO_2, NH_3 and O_3. By varying the proportions of these trace gases and the conditions of radiation of ultraviolet light and temperature, they obtained some detailed information for studying the mechanism of formation of stratospheric sulphate particles. They proposed a chemical model to interpret their laboratory observations and other observed features of stratospheric aerosol. The possible role of nitric acid, however, which is relatively abundant in the stratosphere[2], was not included in their investigation of stratosphere aerosol formation. Also, the possible solid phase of 75% H_2SO_4 by weight in water at −50° C, as discussed by Toon and Pollack[3], was not taken into consideration in their proposed chemical model to interpret the formation mechanism of ammonium sulphate or ammonium persulphate particles in the stratosphere. Toon and Pollack examined some of the physical properties of nitric acid, sulphuric acid and ammonium sulphate in stratospheric thermodynamic conditions[3]. Using physical equilibrium phase diagram analysis techniques[4], they compared the equilibrium vapour pressure over nitric acid solutions with observed water and nitric acid partial pressures in the stratosphere, and concluded that nitric acid cannot be present as an aerosol in the lower stratosphere. For sulphuric acid, they predicted that sulphuric acid aerosol particles in the stratosphere are 75% H_2SO_4 by weight in water, in agreement with observations by Rosen[5]. From the freezing curve of H_2SO_4 solutions[6], Toon and Pollack pointed out that H_2SO_4 (75% in weight)–H_2O (25%) aerosol particles should exist in the lower stratosphere either as a solid or as a supercooled liquid. If most of the stratospheric sulphuric acid aerosol particles are in the solid phase, then the chemical models for the formation of ammonium sulphate using solution chemistry in sulphuric acid would not be applicable.

Here we present an order of magnitude estimate for the equilibrium vapour pressure over the ternary system H_2SO_4-HNO_3-H_2O to study the possibility of stratospheric aerosol formation involving HNO_3. Figure 1 shows the schematic equilibrium phase diagram for the H_2SO_4-HNO_3-H_2O ternary system at −50° C. The percentage labels refer to

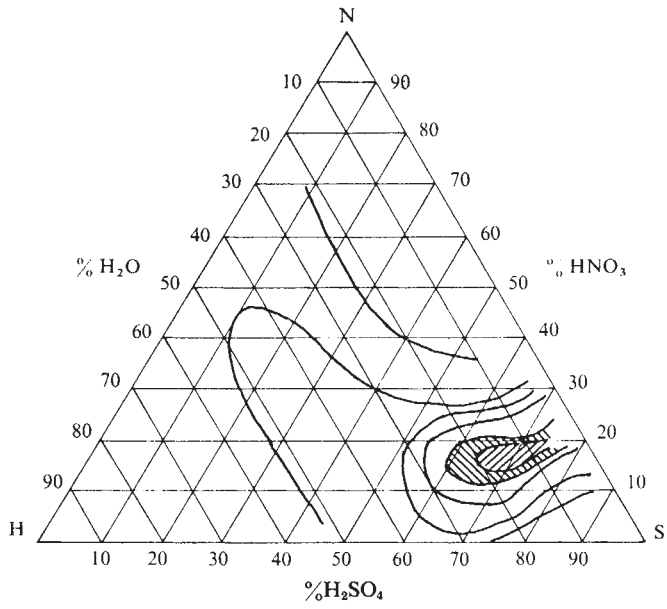


Fig. 1 Order of magnitude changes in vapour pressures for ternary system H_2SO_4-HNO_3-H_2O at −50° C. Vapour pressure curves are obtained by extrapolating from boiling point data. Each contour represents an order of magnitude change in vapour pressure. Note that in reading graph, weight percentages of the three components must add up to 100%. If graph is read incorrectly the sum will be 200%.

the % by weight. The total vapour pressure over the H_2SO_4-HNO_3-H_2O ternary system at −50° C is expressed as a function of the weight composition. The shaded area in the diagram indicates the lowest total vapour pressure region. To estimate the order of magnitude of these equilibrium total vapour pressure we have extrapolated the available data from the international critical tables[7] to −50° C by using the Clausius-Clapeyron equation $dP/dT = L/Tv$, where P is the vapour pressure, T is the absolute temperature, v is the molar volume and L is the latent heat which is assumed to be constant over the temperature range. The vapour pressure obtained by this approximation will be larger than the values measured if the freezing point of these ternary systems is higher than −50° C, since the slope of the curve is proportional to L, and the curves will become steeper at the freezing point. From this crude approximation, we have found a region (Fig. 1, shaded area around 70–80% H_2SO_4, 10–20% HNO_3 and 10–20% H_2O) with the lowest vapour pressure. The vapour pressure in this region is about 10^{-4} to 10^{-5} times lower than the vapour pressure of 75% H_2SO_4 by weight in water. Toon and Pollack estimated the vapour pressure for 75% H_2SO_4 by considering the latent heat as a constant except at the melting point[3] (−33° C). They found the vapour pressure for this composition at −50° C is about 10^{-4} mm Hg. For a supercooled liquid (without considering the freezing point) the vapour pressure for 75% H_2SO_4 at −50° C is about 10^{-3} mm Hg. Therefore, the vapour pressure of the shaded region is about 10^{-8} mm Hg or lower. We base this value on the vapour pressure of 75% H_2SO_4 rather than directly calculating it, because the vapour pressures calculated from ternary system data do not agree with binary system data[7]. This is also the reason why no numerical values for vapour pressure are presented in Fig 1. The curves are intended primarily to indicate the shape and depth of a region where aerosol growth is favoured. The accuracy of the results presented in Figs 1 and 2 depends upon two factors, the boiling point data for the ternary system[7], and the accuracy of the extrapolation to −50° C. We feel that the results are accurate to within an order of magnitude.

For a more accurate estimate of the vapour pressure of this ternary system at −50° C, two factors should be taken

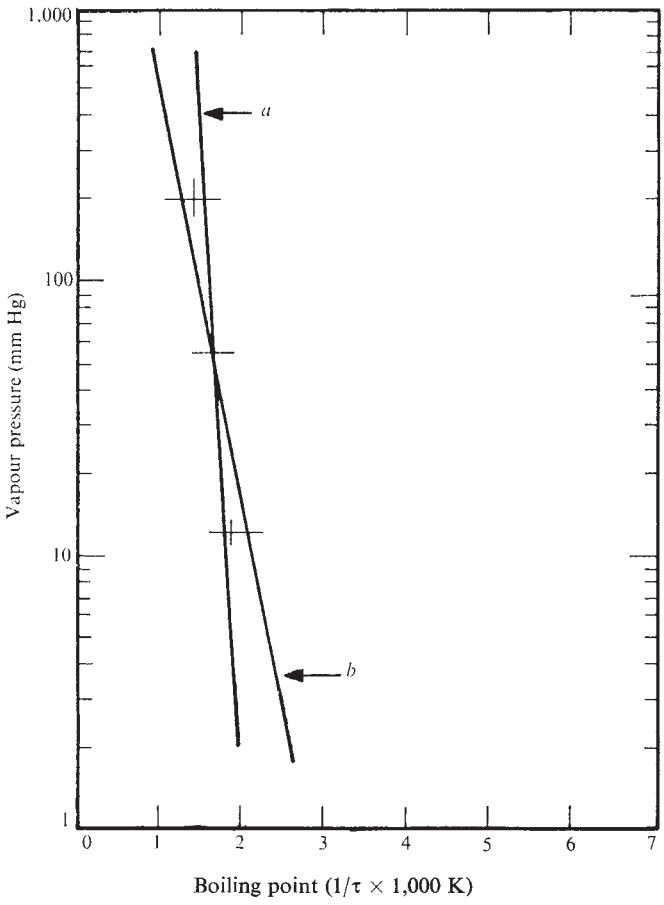


Fig. 2 Experimentally measured vapour pressure points of stratospheric aerosols determined from data obtained by Rosen[4]. Also shown are vapour pressure curves for, *a*, the 75% H_2SO_4–10% H_2O–15% HNO_3 ternary system, and *b*, the 75% H_2SO_4–25% H_2O binary system.

into consideration. First, the latent heat L should be considered to be a function of temperature instead of a constant. In general, the latent heat decreases with increasing T, as it does for water. Then the vapour pressure at 50° C should be lower than what we have estimated. Second, the ternary system in the shaded region may exist as a solid. Since the vapour pressure of a solid is lower than that of a supercooled liquid at the same temperature, once again, our estimate for the vapour pressure could to too high. Therefore, as an order of magnitude estimate, it is probably safe to say that the vapour pressures for the ternary system H_2SO_4-HNO_3-H_2O with weight composition around 70–80% H_2SO_4, 10–20% HNO_3, 10–20% H_2O at −50° C are below the order of 10^{-8} mm Hg.

The measured partial pressure for HNO_3 in the stratosphere at −50° C (refs 2 and 3) is of the order of 10^{-7} to 10^{-6} mm Hg, for H_2O, the measured partial pressure is of the order 10^{-4} to 10^{-3} (ref. 3). Comparing the estimated total vapour pressure of the ternary system H_2SO_4-HNO_3-H_2O with the weight percentage mentioned above, there exists more than sufficient nitric acid and water vapour in the stratosphere to participate in ternary system aerosol formation at −50° C. Therefore, nitric acid should be found in stratospheric aerosols, provided H_2SO_4 is also present. Furthermore, we have compared the vapour pressure of the ternary system with data of stratospheric aerosols as observed by Rosen[5] (Fig. 2). It is found that a ternary H_2SO_4-HNO_3-H_2O system with weight composition 75% H_2SO_4, 10% HNO_3 and 15% H_2O, would conform to the experimentally determined vapour pressure curve as well as the one of 75% sulphuric acid solution fitted by Rosen[5].

Because of the presence of HNO_3, the freezing temperature of the sulphuric acid solution will be reduced. Based on the assumption of ideal solutions, we have estimated the decrease in the freezing temperature caused by adding 10% nitric acid to the sulphuric acid solution (with 75% H_2SO_4 and 25% H_2O). With the heat of fusion of 75% H_2SO_4 taken to be 4,360 calorie mol^{-1} (ref. 8), and the freezing temperature of the 67.5% H_2SO_4, 22.5% H_2O and 10% HNO_3 ternary system is estimated to be −45° C. Since we have a real rather than an ideal solution, we expect the freezing temperature in the shaded region to be lower than −50° C.

We conclude that one cannot rule out the possibility that nitric acid may participate in aerosol formation in the stratosphere. More experimental measurements on the equilibrium vapour pressure for the ternary system H_2SO_4-H_2O or other suitable ternary and multicomponent systems are strongly recommended. Furthermore, it is important that *in situ* measurements to determine whether stratospheric aerosol contain nitric acid are made.

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Potential method of geobarometry using quartz

As crystals grow from solution they may trap small amounts of the solvent and other solutes. It has long been known that minerals evolve gases on heating[1-4] and it has been assumed that, except for alteration during weathering, the gases are incorporated from the growth medium at the time of crystallisation. If the amount and composition of the gases trapped in minerals are to be used to make detailed geological interpretations it is essential that the location of the gas within the crystal structure is established. In minerals like carbonates and hydrates, 'gases' form part of the structure. Sites in which gas can occur include primary and secondary fluid inclusions, point defects, lattice dislocations, grain boundaries, and structure holes. Gas can also be adsorbed on crystal surfaces. Each site has its own characteristic temperature at which gas loss occurs, so that on heating a mineral the gases are released at different temperatures. This provides a method for distinguishing between gases from different sites.

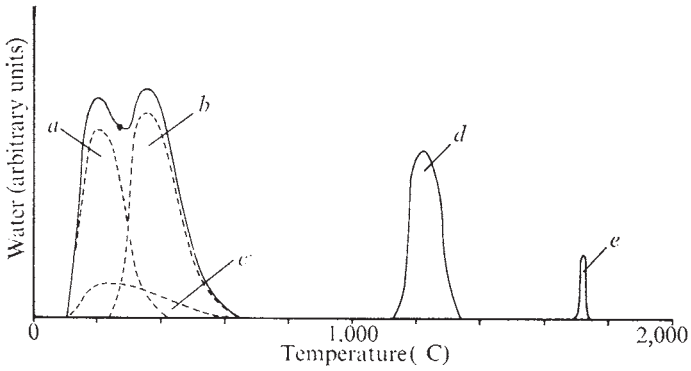


Fig. 1 Water release from quartz as a function of temperature. The solid line indicates the total response. This is made up of separate contributions coming from: *a*, secondary fluid inclusions; *b*, primary fluid inclusions; *c*, surface adsorption; *d*, grain boundaries and lattice defects; *e*, lattice water.

We have studied the location of water in quartz, using a mass spectrometer to monitor the release of water as a function of temperature. Quartz samples (0.5 g) were heated in a vacuum in outgassed mullite or alumina tubes connected to a heated stainless steel gas handling system which formed the inlet to a calibrated mass spectrometer[5]. A resistance-wound furnace was used to attain temperatures of 25–1,250° C, and a gas-torch furnace was used for temperatures up to 1,850° C. All the studied quartz samples showed three major episodes of water release (Fig. 1). In the temperature range 100–450° C water comes from fluid inclusion and from surface adsorption. (The detailed experimental support for this conclusion will be published elsewhere.) The second peak at 1,150–1,300° C is attributed to water released from grain boundaries and dislocations as they thermally anneal[6]. The remaining water is present in point defects and is not released until the quartz lattice is destroyed by melting. No release of water was associated with the α quartz–β quartz (573° C), quartz–tridymite (867° C) or tridymite–crystobalite (1,470° C) transitions. The quantities of water which remain in the liquid when the quartz melts are unknown, but they seem to be insignificant. Infrared studies of the glass produced show no adsorption characteristic of OH or H_2O. As the water vapour released by the sample is continuously pumped away through the mass spectrometer the partial pressure of water vapour over the melt remains low, and because of this the equilibrium amount of water in the melt should also be very small.

Table 1 Conditions of growth for synthetic quartz crystals

Pressure ($N\,m^{-2} \times 10^{-8}$)	Pressure (pound $inch^{-2} \times 10^{-3}$)	Top temperature (° C)	Bottom temperature (° C)	Growth rate ($mm\,d^{-1}$)
1.38	20	349	399	0.67
1.59	23	349	399	1.30
2.14	31	350	396	2.21
2.41	35	352	396	2.26
2.76	40	374	396	2.61
2.90	42	371	410	2.29
3.10	45	360	416	2.64

We have studied seven synthetic hydrothermal crystals of quartz, grown at pressures of 1.38×10^8 to 3.10×10^8 N m^{-2} (45,000 pound $inch^{-2}$), (Table 1), and natural hydrothermal quartz crystals from Brazil and Arkansas. In all cases the release of water as a function of temperature resembled the trend indicated by Fig. 1. As expected, the amount of water in fluid inclusions varied widely, even within a single crystal, but the water which was evolved at about 1,200° C showed a much smaller range. The amount of water released on melting crushed pieces (≈ 3 mm^3) of single crystals of synthetic quartz varied from 1.2 ± 0.2 to 5.8 ± 0.2 cm^3 of water vapour per gram of quartz. Duplicate determinations confirmed the value of 0.2 $cm^3\,g^{-1}$. There is a clear trend of increasing water content with growth pressure (Fig. 2), indicating that the water incorporated into lattice sites is related to the partial pressure of water at the time of crystal growth. The distribution coefficients range from 0.8×10^{-5} to 2.0×10^{-5} ml (STP) per g atom. These are probably minimum values. We have not yet done any experiments to determine the variation of the distribution coefficients with growth temperature or with the composition of the growth medium. It has been reported that the presence of Li^+ in the liquid decreases the total water content of the quartz[7] but the particular location of the water was not investigated. Growth rate varies with growth pressure (Table 1), so there is a trend of increasing water content of the lattice with increasing growth rate. The data are insufficient to resolve completely the influences of pressure and growth rate on the water content. We feel that pressure is the more important because the 2.76×10^8 N m^{-2} (40,000 pound $inch^{-2}$) and 3.10×10^8 N m^{-2} (45,000 pound $inch^{-2}$) crystals were grown at the same rate and have very different water contents. Also, the 2.14×10^8 N m^{-2} (31,000 pound $inch^{-2}$), 2.41×10^8 N m^{-2} (35,000 pound $inch^{-2}$), and 2.90×10^8 N m^{-2} (42,000 pound $inch^{-2}$) crystals were grown at similar rates but have different water contents, which vary as a function of growth pressure.

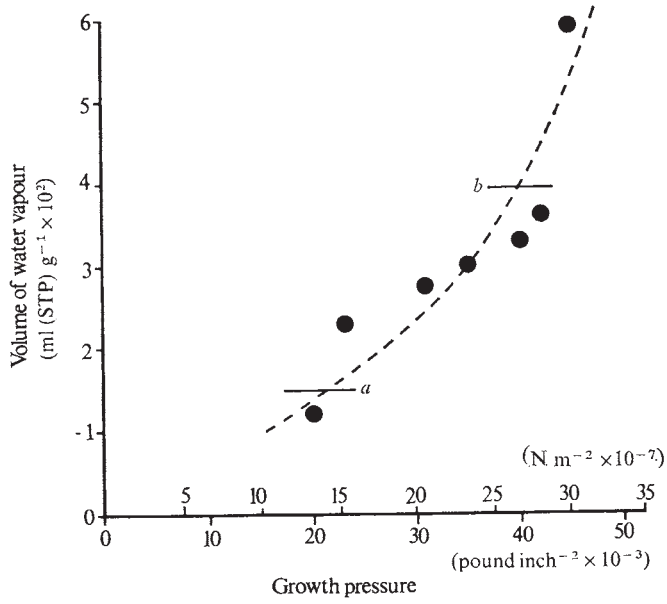


Fig. 2 Amount of water evolved from natural and synthetic quartz crystals between 1,650° C and the melting point, as a function of growth pressure. Values plotted at 1.38×10^8 N m^{-2} (20,000 pound $inch^{-2}$) and 3.10×10^8 N m^{-2} (45,000 pound $inch^{-2}$) are averages obtained from duplicate analyses. *a*, *b*, Water values obtained for the Arkansas and Brazilian quartz, respectively.

In most hydrothermal systems the partial pressure of water is approximately the same as to the total pressure, so a determination of the amount of water released at the melting point permits an estimate of the pressure at the time of crystal growth (assuming that the distribution coefficient is not appreciably altered by temperature, growth rate or composition of the growth medium). We have applied this potential method of geobarometry to the Brazilian and Arkansas quartz crystals, and it indicates pressures of 2.76×10^8 N m^{-2} (40,000 pound $inch^{-2}$) and 1.48×10^8 N m^{-2} (21,500 pound $inch^{-2}$) respectively. These values correspond to depths of 12,200 m (40,000 feet) and 6,550 m (21,500 feet), respectively, if the lithostatic pressure gradient has its usual value of 2.26×10^4 N m^{-2} m^{-1} (1.0 pound $inch^{-2}$ $foot^{-1}$). For a typical continental geothermal gradient of 30° C km^{-1} these depths correspond to temperatures of 386° C for the Brazilian quartz, and 222° C

for the Arkansas quartz. If these temperatures and pressures are used to define a line of constant specific volume on a water phase diagram then the temperature at which the specific volume on a water phase diagram then the temperature at which the specific volume line intersects the two-phase boundary corresponds to the filling temperatures of the fluid inclusions. The calculated filling temperature for the Arkansas quartz is 138° C, which is in good agreement with the observed values of 100–150° C (R. H. Konig, personal communication). For Brazilian quartz, filling temperatures ranging up to 190° C have been reported[8,9]. The calculated value is somewhat high at 213° C. The calculated values were, however, obtained from the phase diagram for pure water and so they will be slightly higher than those calculated using data for saline solutions.

If the water content of the quartz lattice gives the partial pressure of water in the growth medium then this technique could probably be used with magmatic quartz to give the partial pressures of water in silicate melts. This method of determining partial pressures could also be extended to other minerals and to other gases if synthetic samples are available for calibrating the gas content as a function of the partial pressure in the growth medium.

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Fronts in the Irish Sea

A MARKED discontinuity in the sea surface temperature with horizontal gradients up to 1° C km^{-1} is often observed in the western Irish Sea during the summer. This feature is the boundary between stratified and vertically mixed regimes[1]. Here we describe observations by airborne radiation thermometer (ART) and conventional methods, and suggest a simple model accounting for the observed form and stable position of the front.

The region of low tidal energy to the west of the Isle of Man (IOM), (see Fig. 1) permits stabilisation of the water column by insolation during the early part of the summer. At the beginning of June stratification of this area is usually well established, and a rapid warming of the surface waters ensues. This leads to a sharp temperature discontinuity at the surface, which may involve changes as large as 3° C in June and July. The temperature contrast is generally diminished in August as the temperature of the mixed regime approaches that of the surface layer in the stratified area. Strong horizontal temperature gradients remain, however, further down in the water column.

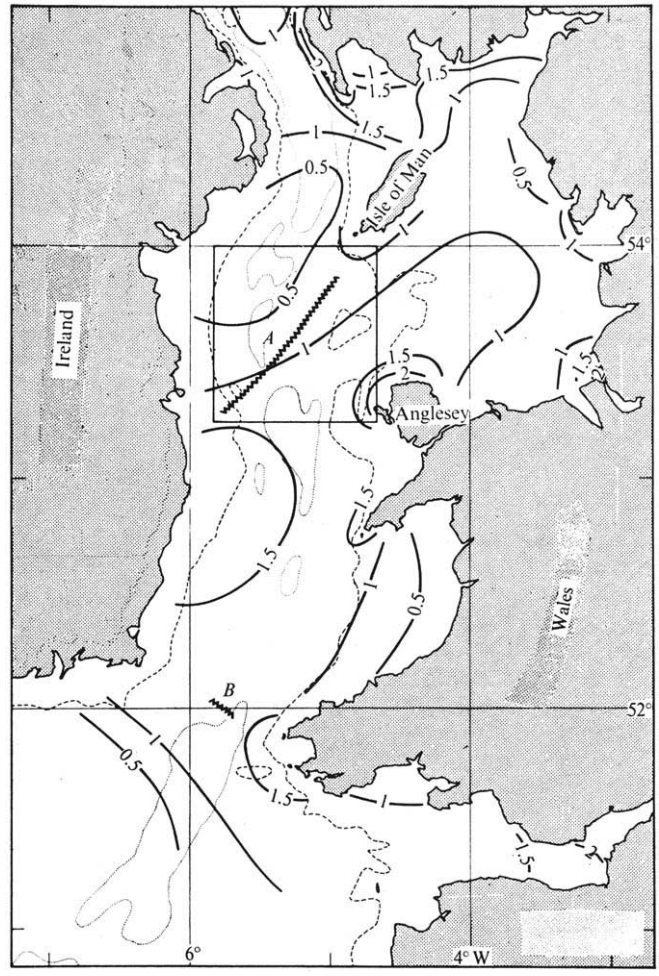


Fig. 1 Amplitude (m s^{-1}) of the discussed tidal streams (solid lines) in the Irish Sea at mean springs from Bowden[3]. *A*, *B*, (heavy zigzag line), Observed positions of fronts. Depth contours are 100 m (dotted) and 50 m (dashed).

The position of the maximum surface temperature gradient, deduced from surface temperature surveys, is shown in Fig. 2. Surveys *a*–*e* are based on thermograph data supplemented by TSD stations. Coverage is limited as a result of the ship's slow speed (about 10 knots) and significant evolution of the temperature field resulting from surface heating and wind mixing may well have occurred during the survey. These difficulties preclude investigation of the detail form of the front from ship's observations alone, and we therefore proceeded to obtain alternative data using an ART. Surveys *f* and *g* (Fig. 2) represent results of ART surveys. In these two cases the position of the front has been corrected for tidal movements on the basis of the best available tidal stream data for the area. Errors which occurred in the earlier surveys because of tidal displacements, should not exceed 10 km.

The position of the front does not vary greatly (Fig. 2). With the exception of survey *c* all the observed positions lie within 10 km of a mean position. The front observed on this occasion was not well defined, and may have represented a transient condition during the establishing of stratification.

In aerial survey work, the radiation temperature of the sea surface is measured using a portable radiation ther-

mometer fitted to an aircraft. The instrument is calibrated in flight with a constant temperature black body, and simultaneous measurements of the true sea surface temperature at a depth of 3 m are made from a ship at selected points on the flight plan. With these controls the errors in the final estimate of sea surface temperature may be kept as low as ± 0.1° C. The aircraft operates at an altitude of approximately 150 m, from which the radiation sensor effectively samples an area of 50 m² of the surface of the sea.

Navigation is accomplished using the Decca Chain 3B/MP, with the operational legs flown approximately north–south along red lanes at intervals of ~2 km (3 Decca lanes). A tidal correction is applied to the data, which are then transformed on to a square grid with a mesh size of 2.54 hm. This is accomplished for each grid point by meaning all the temperature values falling in a square of length 3.81 km which is centred on that point. The resultant smoothing helps to reduce the noise inherent in the raw data. Objective contouring is then accomplished by linear interpolation between adjacent grid points.

Figure 3*a* is the result of an ART flight on June 5, 1973. It shows a well developed frontal situation extending for almost 50 km through the survey area. The flight was made under nearly ideal conditions (clear sky, wind less than 10 knots) following a long period of almost calm weather. In the three days before the flight, the wind at Ronaldsway (IOM) did not exceed 15 knots and no winds greater than 20 knots were reported during the preceding two weeks. It therefore seems reasonable to assume that the observed position of the front represents an equilibrium undisturbed by wind mixing and transient motions produced by wind stress.

The relative stability of the front is also indicated by observations made by the aircrew during the flight. The pilot reported that the line of the front was clearly visible because of an accumulation of surface material in the vicinity of the maximum surface temperature gradient. The aircrew also noted changes in the colour of the sea and in the state of the sea as the front was crossed. The change in colour (greener in the mixed water, bluer in the stratified region) is probably a manifestation of the changes in the standing crop of phytoplankton in the frontal region. Estimates made in July 1971 indicate that the chlorophyll concentration decreases from ~1.5 μg l⁻¹ in the mixed water to ~0.5 μg l⁻¹ in the surface water of the stratified

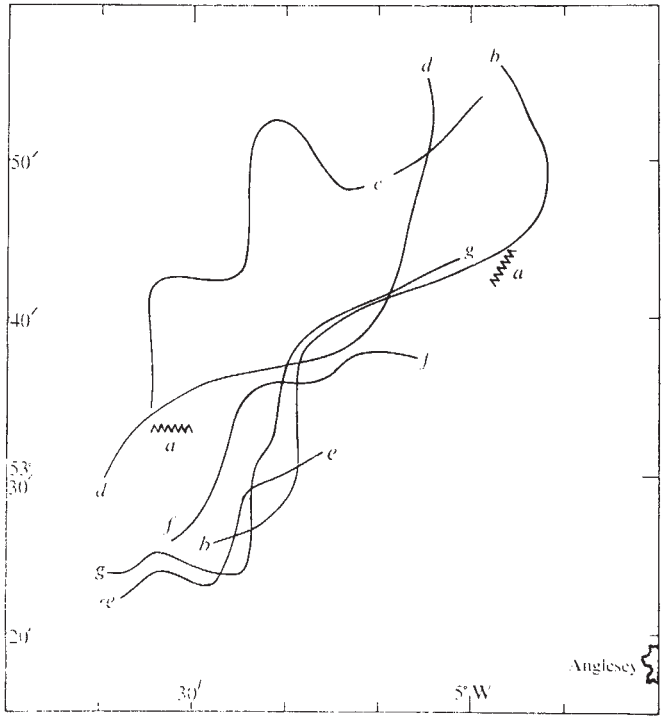


Fig. 2 Position of the maximum temperature gradient in the boundary front *A* as deduced from sea surface temperature surveys. Date of measurements: *a*, August 18–21, 1969; *b*, June 15–19, 1970; *c*, June 7–11, 1971; *d*, July 12–15, 1971; *e*, June 26–30, 1972; *f*, August 24, 1972; *g*, June 5, 1973. *a*, *b*, *c*, *d*, and *e*, thermograph and TSD data; *f*, and *g*, ART data.

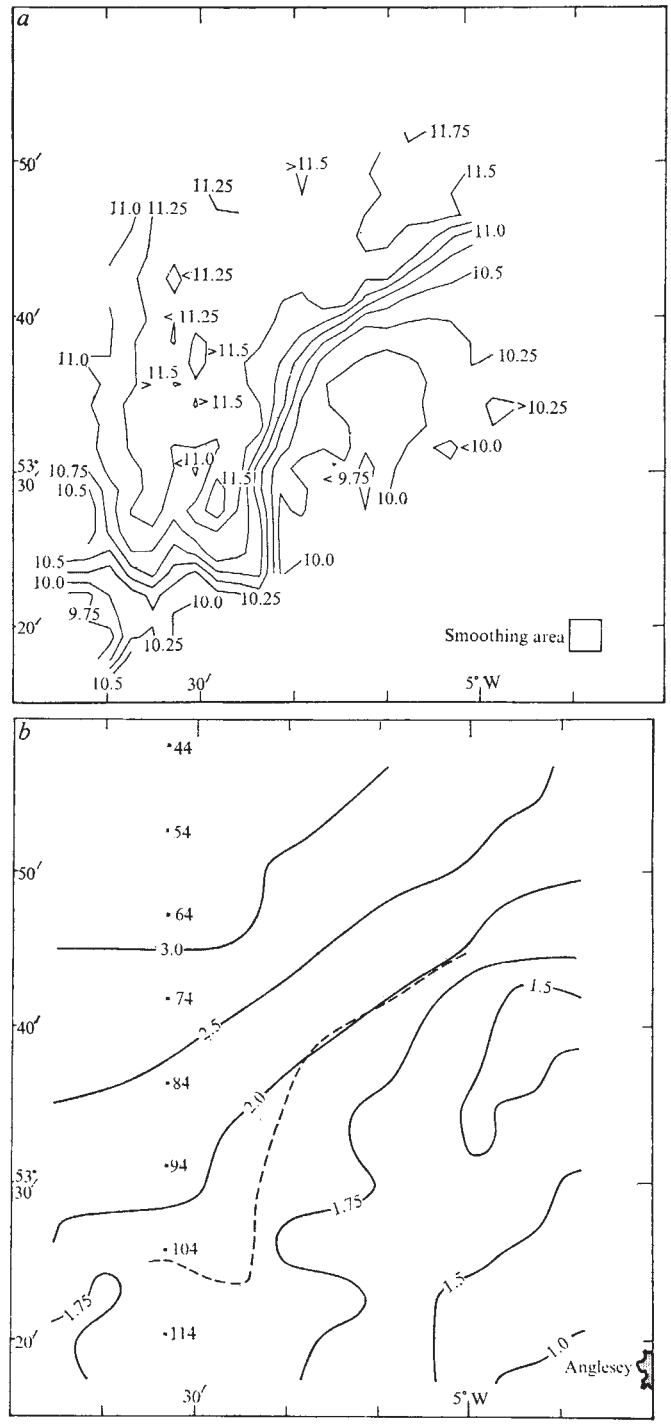


Fig. 3 *a*, Sea surface temperature distribution for June 5, 1973. Contours are at intervals of 0.25° C and have been drawn by an objective contouring programme. The data have been corrected for the observed difference between the radiation and thermograph temperatures of the sea surface. *b*, Contours of $\log_{10} h/u^3$ with h in metres and u in m s^{-1}. The dashed line shows the position of maximum gradient in Fig. 3*a*. The station positions shown (black squares) refer to the section shown in Fig. 4, which was worked in August 1970.

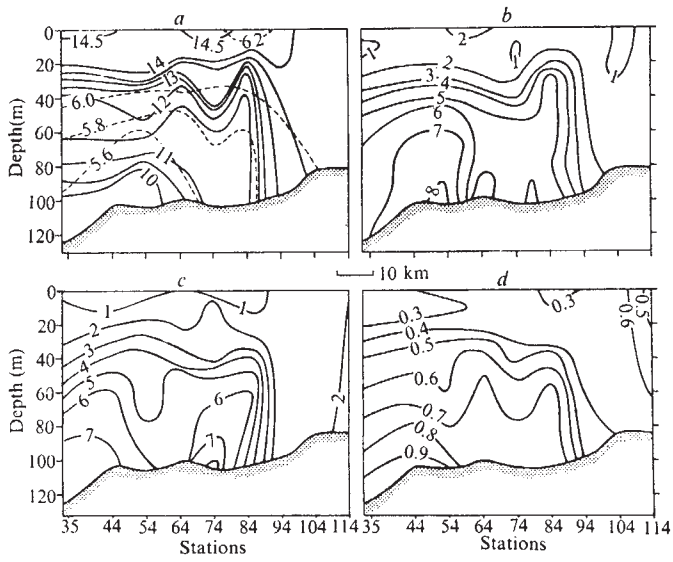


Fig. 4 North–south section across front *A*, showing the distribution of: *a*, temperature (°C), (solid line), and oxygen (ml l^{-1} at NTP), (dashed line); *b*, silicates (μg atom silica l^{-1}); *c*, nitrate (μg atom nitrate nitrogen); *d*, phosphates (μg atom phosphate phosphorus). The positions of the stations are shown in Fig. 3*b*, Data taken August 17–20, 1971.

region. These changes in the standing crop of phytoplankton may be associated with limitations which are imposed by the availability of nutrients in the two regimes.

The distribution of nutrients in a section approximately perpendicular to the front is shown in Fig. 4 together with the corresponding temperature and dissolved-oxygen data. These results were obtained in mid-August 1971 but are representative of the distribution of nutrients throughout the summer months. The contrast in surface temperature is slight, but the front is clearly apparent in the intensified horizontal gradients below the surface mixed layer between stations 84 and 94. The distributions of all three nutrients, nitrate, phosphate and silicate, and the dissolved oxygen, are strongly correlated with the temperature structure. In the cases of nitrate and phosphate, the concentration in the mixed water (stations 104, 114) is generally higher than in the surface layer of the stratified regime, but large concentrations of all of the nutrients remain in the bottom water of the stratified regime, which is also characterised by depletion of the dissolved oxygen content. The higher values of nitrate and phosphate at the surface in the vertically mixed regime probably result from replenishment of nutrients from the bottom, which is not possible in the stratified area because vertical mixing is strongly inhibited.

The relative consistency of the observed position of the front suggests that the transition between stratified and unstratified regimes is essentially controlled by the level of tidal mixing. To examine this hypothesis we have considered the energetics of tidal mixing in a simple case.

Suppose that an amount, q, of heat is introduced into the surface of a water column of depth h, and an initial density ρ. This heat input produces a density change $\Delta\rho$ in a thin surface layer Δh. To mix the column vertically to a uniform density ρ' in a depth h' the potential energy must be increased by an amount V_2-V_1 where

$V_1=\frac{1}{2}\rho gh^2-\Delta\rho\Delta hgh+Ph$=potential energy before mixing

$V_2=\frac{1}{2}\rho' gh'^2+Ph'$=potential energy after mixing; where P=atmospheric pressure.

Assuming that the density is a linear function of temperature, and neglecting salinity as a source of buoyancy input, then it is easily shown that $h=h'$ and

$$V_2-V_1=\alpha qgh/2c$$

where α is the linear expansion coefficient and c is the specific heat. For a rate of heat input Q, the demand for potential energy to maintain mixing would be

$$dV/dt=\alpha Qgh/2c$$

Energy is lost from the tidal motion at a mean rate of

$$dE/dt=\overline{\tau_b u_b}=k\rho\,|\overline{u^3{}_b}|=(4k/3\pi)\rho\ u^3{}_o$$

where μ_b and τ_b are the velocity and shear stress respectively, near the bottom, and u_o is the amplitude of u_b. k is a constant in the assumed quadratic friction law. A fraction ε of this kinetic energy is considered to be available for increasing the potential energy of the water column so that at transition there is a balance defined by

$$h/u^3{}_o=8cpk\varepsilon/3\pi\alpha gQ$$

If the area and time of interest are limited, Q can be regarded as constant. Then, providing ε and k are also constants, the locus of the front should be defined by a critical value of h/u^3. Assuming that u_o is proportional to the observed surface tidal velocity amplitude u_s, h/u_s^3 can be used as the parameter which controls the formation of a front. Results for h/u_s^3, based on the computations of a numerical model[2], are shown in Fig. 3*b*, along with the line of the front observed in June 1973. The front is approximately parallel to the contours of h/u_s^3 at a value between 65 and 100.

Assuming that ε and k are constant, this approach can be used to predict the occurrence of stratification and fronts for other parts of the continental shelf where the appropriate value of Q is known. As a more exhaustive test of our hypothesis we have a programme in hand to compare all the available data on stratification on the shelf, with the distribution of h/u_s^3. We have already examined a front in the south-western approaches, the position of which (front *B*, Fig. 1) was predicted in this way. Observations in August 1972 revealed a transition occurring for $h/u_s^3\simeq 55$. Considering the uncertainties in u_s this value is not significantly different from that given for the *A* front. Provisional data for a less well defined transition in the Channel suggest a similar critical value of h/u_s^3

At present, relatively little is known of the residual velocity field in the vicinity of the front, largely because of the difficulties of making measurements of a velocity field which is being displaced tidally through an excursion of about 10 km. Observations with parachute drogues, which were tracked by radio in both the *A* and *B* fronts, show residual velocity vectors with speeds up to 10 cm s^{-1} approximately parallel to the front. Whether or not these velocities are generally in geostrophic balance with the density field remains uncertain on the basis of present knowledge. Such a balance is, however, suggested by data obtained in observations of the *B* front in August 1972, and if confirmed, would indicate longitudinal velocities of up to 50 cm s^{-1} in parts of the front.

We are grateful to Graham Savidge of the Marine Science laboratories for the nutrient data in Fig. 4 and to the Meteorological Research Flight for providing the ART facilities.

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Irregularities in dendrochronological calibration

MOST of the 'wiggles' in the Suess curve[1], which is used for calibrating radiocarbon dates (denoted b.p.; sidereal dates denoted BP) in terms of dendrochronology, are likely to be artefacts[2]. Curves with almost undiscernible fluctuations have been produced. There are, however, physical reasons for expecting some changes in the ^{14}C content of the atmosphere to have occurred and it is important to locate them by a method that does not depend either on intuition or on minimising deviations over the whole range of the curve[4-6].

It should be possible to locate wiggles, if they exist, by using the estimated radiocarbon ages of samples themselves. If the samples have a uniform age distribution in sidereal time, then the apparent distribution in radiocarbon years would be uneven around the date at which the wiggle occurs; in other words, the apparent distribution of the samples in radiocarbon years would act as an indicator of the first differential of the slope of the calibration curve. Simulation trials showed that the uneven distribution can indeed be picked up, particularly if the sample ages are grouped at intervals which are small in comparison with the expected duration of the wiggle, but that the method is much more likely to be successful with a short lived sinusoidal irregularity than with a gradual but permanent change in slope. Stuiver (see ref. 7) has apparently tried a similar idea but his results are not available to us.

If the method is to be successful, there have to be enough sample ages available to ensure that, first, their distribution is uniform (although not necessarily constant) and, second that small regions of local high frequency are swamped. To meet these requirements we have considered all of the radiocarbon

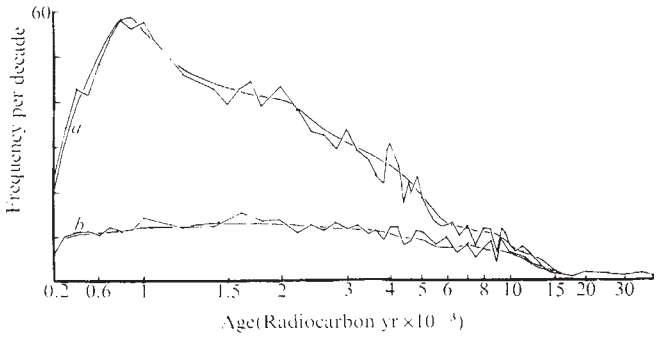


Fig. 1 Frequency distribution of published radiocarbon dates. *a*, All dates; *b*, geological dates. In each case a smoothed curve has been constructed (see text).

dates recorded in volumes 1 to 15(2) of the journal *Radiocarbon*. All of the dates used had the half life of 5,570 yr; deep sea samples were omitted but pelagic shell samples were not, because there is some doubt about whether any correction is necessary for shallow waters[8,9]. No attempt was made to separate samples from the southern hemisphere. 'Archaeological and geological dates were collected separately. We recorded approximately 13,800 archaeological, and 12,500 geological, dates going back 50,000 radiocarbon years.

The archaeological dates are not so suitable for testing, because almost all occur in the first 4,000 yr b.p. They will be discussed elsewhere, and here we report findings from the geological dates, and from the combined series, going back to 40,000 yr b.p.

A smoothed frequency curve was constructed by applying first-order smoothing algorithms (IBM SSP routines SE13 and SE15) successively to the grouped totals for intervals of 200 yr. From this a Kolmogorov Smirnov statistic was calculated and was used to provide confidence limits[10] for a curve corresponding to at least 150 dates, or extending over at least 150 yr. The sections were made to overlap, so that each decade interval had more than one chance of being in the centre of the sectional

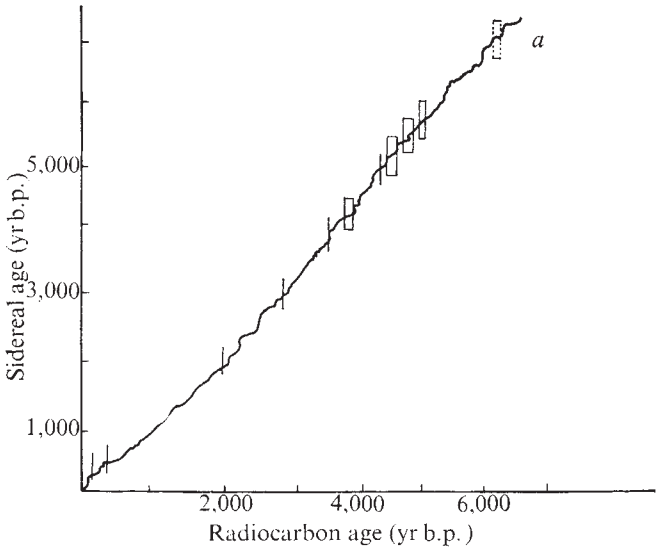


Fig. 2 Correlation of wiggles predicted by frequency analysis with MASCA calibration curve[12] *a*, geological series.

curve. The actual frequencies of the dates, grouped in decades, were then tested against this smoothed curve, using 1%, 5% and 10% probability criteria[10].

It was possible to identify several probable wiggles. Beyond 14,000 yr b.p. the frequency of the dates is too low (Fig. 1) to pick up with certainty anything other than a possible irregularity at about 29,000 yr b.p. This correlates moderately well with the shift in the $^{18}O/^{16}O$ ratio reported from the Camp Century ice sheet between 30,000 and 35,000 yr BP[11]. Excluding irregularities which extend over just a single decade, only five wiggles are significant at the 1% level: they occur in two major groups at about 3,800, 4,400 and 4,700 yr and 8,800 and 8,900 yr b.p. The archaeological data, at a low significance level, indicate two wiggles, at 200 and 400 yr b.p., which may be assigned to the original Suess and de Vries deviations. There is quite a good correlation between the irregularities predicted by this study and the small scale variations (which are nevertheless important for archaeological dating) in the recently published MASCA dendrochronological calibration curve (Fig. 2). The chief difference is the almost complete absence of wiggles in the third millenium BC.

There is also a remarkably good correlation between the irregularities found in this study and Bray's[13] estimates of the dates of maximum glacial retreat (we assume that his dates are uncorrected radiocarbon years). It is therefore likely that the most intense and long lasting wiggles predicted by us—at about 8,600–9,000 yr b.p.—correspond to the large climatic change at the beginning of the present interglacial. This could be used to try to obtain an absolute dating for this irregularity. If the slope of the dendrochronological calibration curves from 1,000 to 5,000 yr BP were to continue back indefinitely, the period 8,600 to 9,000 yr b.p. (radiocarbon) would correct to 10,500–11,000 yr BP. There is some difficulty in obtaining estimates of climatic events which do not depend on uncorrected radiocarbon dating. The oxygen isotope profiles published by Johnsen *et al.*[11] show that the Allerod change was completed by about 11,000 yr BP in the Greenland ice core, which fits rather well with our extrapolated correction. On the other hand, Stuiver's curve relating the age determined by the ^{14}C method to varve counts[7], shows a large deviation in both Swedish and Minnesota varves at about 8,000–8,500 yr BP. If these are sidereal dates, identical with the irregularity under discussion, the dendrochronological correction would be zero at about this time. On this graph, however, the Swedish varves show absolutely no shift in $\Delta^{14}C$ over the period from 8,500 to 12,500 yr BP (ref. 14), which does not seem to correlate with the $^{18}O/^{16}O$ record at

Table 1 Significant irregularities (radiocarbon yr) in frequencies of geological ^{14}C dates

Date b.p.	Significance level	Date of temperature maximum (from Bray[13])
890	$P<0.1$	900
1,620	0.05	1,600
3,180	0.1	—
3,350 – 3,390	0.1	3,300
*		3,800
4,200 – 4,210	0.01	—
4,460 – 4,470	0.05	4,400
—		4,500
4,660 – 4,690	0.05	—
—		5,800
—		5,900
6,000, 6,060		—
6,550 – 6,640	0.05	6,500
†		7,100
8,600 – 8,800	0.01	8,500
8,910 – 9,050	0.01	8,800
—		9,600
—		9,700
10,140 – 10,190	0.05	—
10,990	0.05	11,110 —
11,980 – 12,020	0.05	11,800
		12,250
12,590 – 12,640	0.1	—
—		13,400
—		14,100
14,640 – 14,790	0.1	14,700 – 15,500
		16,100, 16,800
—		18,000, 18,300
		19,100, 20,300
27,430 – 27,490	0.1	—
27,630 – 27,660	0.1	—

Estimates of significance are conservative (see ref. 10).
* Irregularity at 3,770 – 3,810 yr b.p. in combined data ($P < 0.01$).
† Irregularity at 6,910 – 7,020 b.p. in archaeological data ($P < 0.05$).

all. Moreover, this interpretation of the Swedish data has been disputed[15]. At present, we prefer to correlate our chart of irregularities with the oxygen isotope profile, partly because of the coincidence at 30,000 yr BP and (with the Southern Hemisphere profile) at 4,000 yr BP, and partly because a massive ^{14}C correction factor would explain the present dearth of dates for the Mesolithic in the period 8,000 to 10,000 yr BP (J. B. Campbell, personal communication). This identification is very tentative.

Nonparametric search techniques require a point estimate for each event that forms part of the frequency, whereas radiocarbon determinations, by their nature, include a statistical uncertainty. We are aware that in converting from one to the other we have lost the information contained in the estimate of standard deviation accompanying each published date, but it was inevitable that many different kinds of sample, with different inherent errors, should be brought together, if enough dates were to be assembled to cover adequately a long time-span. No single laboratory could have provided enough dates for this purpose. We feel that our approach was justified in that, up to about 7,000 yr b.p. (after which archaeological dates become very infrequent), there is substantial agreement between the dates of wiggles predicted by the geological and archaeological dates taken separately, particularly in the period of intense disturbance from 4,000 to 5,000 yr b.p. It is likely that some smaller wiggles may have become blurred, but in any case we do not think that the method described here would pick up the recently reported decadal rhythms. We wished in the first instance to see whether independent evidence could be provided for any of the fluctuations of about a century's duration, which characterise the Suess curve. For a similar reason we included dates from both the Old World and the New World, and from both hemispheres. There has been an increasing tendency for archaeologists to point out that the dendrochronological evidence for ^{14}C calibration is at present derived from a relatively small area of the United States, and to draw attention to apparent discrepancies in certain millenia and in certain areas of the Old World (for example, the Mediterranean basin). It therefore seemed important to establish 'global' wiggles, although it would undoubtedly be interesting in later studies to test separately dates from all quadrants of the earth.

We are satisfied that there are about four serious wiggles in the dendrochronological calibration curve, concentrated in the second and third millenia BC. It should not be difficult to determine the shapes of the irregularities by standard statistical techniques, now that the regions in which to search have been identified.

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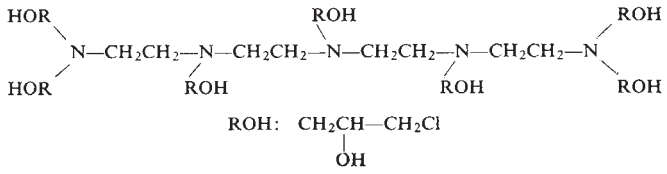
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Polymer structures and turbulent shear stability of drag reducing solutions

THE phenomenon of frictional drag reduction in the turbulent flow of Newtonian fluids, by the addition of high molecular weight polymers, has been known for some time. Because of the great potential it offers in various technological applications this phenomenon has generated considerable engineering interest. Unfortunately, when a limited volume of polymer solution is continuously exposed to mechanical shearing action, either by repeated use[1] or by passing through long pipelines[2], drag reduction rapidly declines, indicating a rapid breakdown of the polymer molecules[3]. This polymer degradation greatly limits the application of drag reducing polymers. When considering the use of high molecular weight polymers in operating systems their shear stability must be considered as equally important as their effectiveness in drag reduction. Polymer degradation effect is likely to be caused by the scission of molecular entanglements or the breaking of individual molecules induced by the shear stresses associated with very high local shear rates[4]. But previous studies were made mainly with linear polymers such as polyethylene oxide (PEO) and polyacrylamide (PAM), because a linear structure was believed to be most effective[5]. We have studied the effect of polymer structure on turbulent drag reduction by synthesising drag-reducing agents of different structures[6]. Here we present the results of an investigation of the degradation behaviour of a highly branched

PAM, and compare the results with those observed with ordinary linear polymers.

The branched PAM sample was synthesised by grafting PAM chains on to a small, nucleus molecule. The nucleus was prepared by reacting tetraethylene pentamine with epichlorohydrin. A typical structure of this backbone molecule may be represented as follows:



There was a total of seven potential chain-grafting sites in this molecule. Through the redox reaction of the molecule with ceric ion, the graft polymerisation of acrylamide was initiated on the ROH groups[7]. The polymerisation started instantaneously and proceeded at a much higher rate than with the ceric ion alone. This suggested that the probability of ungrafted acrylamide polymerisation occurring is negligibly small. The polymerisation was carried out in aqueous solution using the redox system under a nitrogen atmosphere for 5 h at 32° C, resulting in a gel-like, transparent material which was completely water-soluble. The intrinsic viscosity of this PAM compound was 6.13 dl g^{-1}, leading to an estimated molecular weight of 1.7×10^6 (using the relationship in ref. 8). But as this relationship was developed for linear PAM, it may not be relevant to the present highly branched structure. The details on the polymerisation will be published elsewhere.

The drag-reducing capability of the branched PAM sample was characterised in a capillary flow system of diameter 0.1575 cm. A detailed description of the apparatus was presented elsewhere[9]. Using the characterisation technique developed

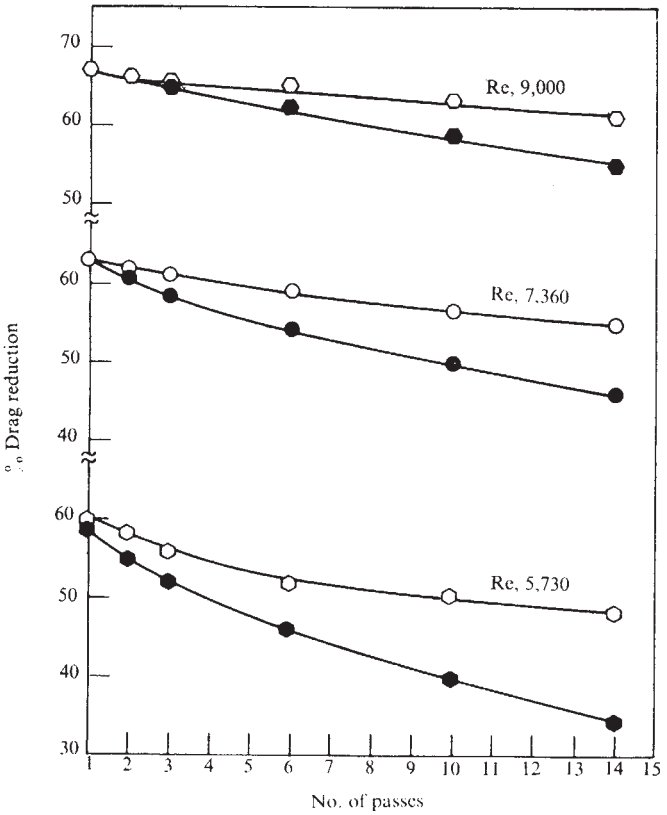


Fig. 1 Degradation behaviour of the 100 p.p.m. solutions of PEO (closed symbols) and branched PAM (open symbols).

earlier[10], the effectiveness of the branched PAM was evaluated at a fixed Reynolds number of 9,000. On a unit concentration basis at infinite dilution this compound had an effectiveness measure (*Eff*) = 4.14 in units of percentage drag reduction per parts per million (% / p.p.m.). The degradation behaviour of commercial linear PEO and PAM compounds with similar effectiveness was compared with that of the branched PAM. Polyox-WSRN-3000 (Union Carbide Corp.; *Eff* = 4.15 % / p.p.m.) and Magnifloc 905N (American Cyanamid Co.; *Eff* = 3.78 % / p.p.m.) were used. The solutions were repeatedly passed through the capillary and the change in percentage drag reduction recorded for each pass. These measurements provided

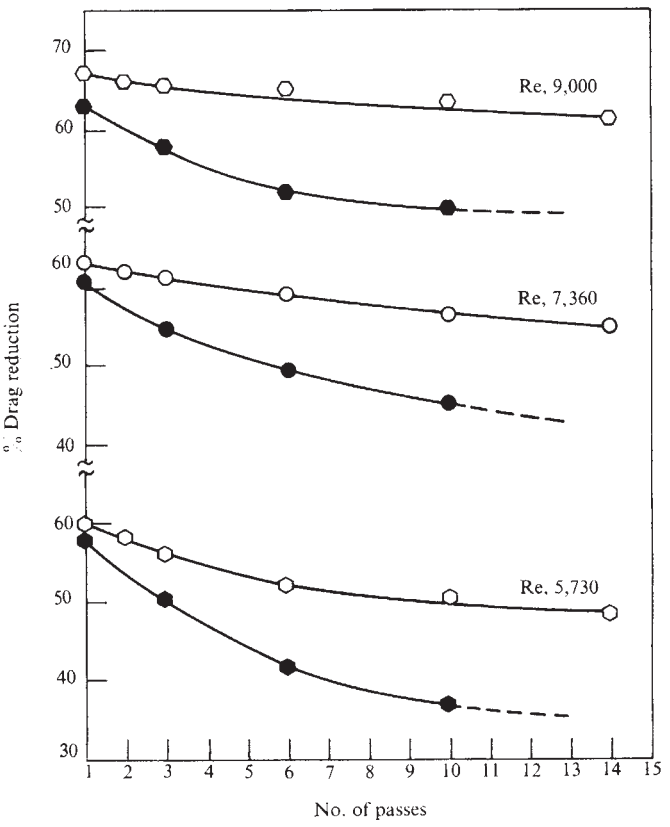


Fig. 2 Degradation behaviours of the 100 p.p.m. solutions of linear PAM (closed symbols) and branched PAM (open symbols).

a relative measure of the shear stability of these compounds of different molecular structure.

The degradation behaviour of 100 p.p.m. solutions of the branched PAM, the linear PAM and PEO are shown in Figs 1 and 2. The data show comparisons on an equal concentration basis with approximately equal initial percentage drag reduction. At three different Reynolds numbers, the solutions of linear PAM and PEO showed a very rapid decline in percentage drag reduction with increasing number of passes through the capillary. The rate of the decrease in percentage drag reduction is much slower for the branched PAM. Such a contrast in degradation behaviour clearly demonstrates the superior shear stability gained by molecular branching. Physically, this may be explained by the molecular scission concept. Recent experimental results of a degradation study of polyacrylamide in a high shear flow[11] showed general agreement with the theoretical prediction of Bueche's midpoint break theory[12]. According to this theory, polymer chain breaking does not take place at random along the chain but occurs predominantly in the central portion of the chain where the extending forces are maximum. In the case of a linear polymer, this kind of chain breakage will immediately reduce the polymer molecular weight to approximately one-half. The drag reducing ability of

the compound is therefore greatly reduced because of the strong dependence of drag reduction on polymer molecular weight[6]. In the case of the branched PAM sample, however, the reduction in molecular weight is apparently less drastic probably because the shear forces acting on the polymer are distributed among the individual chains. Any reduction in molecular weight, when it occurs, will probably be of the order of a chain length rather than one-half of the molecule.

It can be seen that the improvement of the shear stability by branching is more pronounced at lower Reynolds numbers. If turbulent drag reduction is considered as a manifestation of the intensive interactions between polymer molecules and the dissipative turbulent eddies, this Reynolds number effect may be explained as follows. In the dissipation range, the Komolgorov eddy scale, η, is related to the kinematic viscosity ν and the dissipation ε by $\eta = (\nu^3/\varepsilon)^{1/4}$. It can easily be shown that[13]

$$\eta/d \sim (\nu^3 d/u^3)^{1/4}/d = (\nu/ud)^{3/4} = Re^{-3/4}$$

or

$$\eta \sim d/Re^{3/4}$$

where d is the capillary diameter and Re the Reynolds number. Since d is fixed in the present case, the turbulent dissipative eddies have relatively smaller scales at higher Reynolds numbers than those in flows of lower Reynolds numbers. The degraded polymer molecules, presumably having smaller coil sizes, may still be able to interact effectively with the dissipative eddies in flows of higher Reynolds numbers, but have become less effective in lower Reynolds number flows.

The present results also suggest that the linear PAM solution shows as poor shear stability as the PEO solution. This is in contrast to the general belief that the PAM solution is more shear stable than the PEO solution in turbulent flows. From the chemical point of view, a better shear stability for PAM can be possible only if some turbulence-induced oxidation is involved in the degradation process, as proposed by White[14], but this is not obvious according to the present data. We showed that the presence of counter-ions surrounding the domain of an ionic polymer could greatly improve the polymer shear stability in turbulent flow[15]. Most of the previous PAM degradation studies used commercially manufactured compounds. These materials, after long handling and storage periods, could very possibly have become partially hydrolysed to form copolymers of PAM and polyacrylic acid. This is especially true if the materials were exposed to moisture for a long time. This variation, as we have shown[15], could very well introduce higher resistance to mechanical shearing to these so-called PAM compounds.

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Hardening of immersed metals by ultrasound

During electrodeposition in an ultrasonically agitated solution, the hardness of copper anodes increases, and sometimes they perforate in a nonuniform manner[1]. Deposits plated from electrolytes which have been subjected to ultrasound have a higher hardness than those produced from still solutions[2,3]. Here we report on the change in hardness of a wide range of metals.

We used strips of metal about 10 cm long, 1 cm wide and 0.1 cm thick. The majority of these were annealed for 2 h at a temperature of about 60% of the melting point (in degrees absolute) and the hardness (HV) was measured with a microhardness tester using a load of 25, 50 or 100 g. The same load was used in all the measurements of any one metal.

The metal strips were subjected to ultrasound by immersing them in tap water in an ultrasonic tank. The specimens were treated individually to prevent shielding and were clamped centrally and perpendicularly about 1 cm above a 12.5 cm diameter transducer probe. The ultrasonic field, of frequency 13 kHz and intensity 930 W m^{-2}, was applied for 5 min. The specimens were then removed and the hardness of an area in the centre of the strip was measured immediately. This procedure was repeated keeping the metals in the ultrasonic field for increasing intervals of up to a maximum of 1,500 min. Tin and zinc, however, perforated before the end of this time. The hardness was measured again one month after the completion of the experiment to check whether any change had occurred during storage at room temperature.

The changes in the hardness of the various metals during the time of ultrasonic bombardment are shown in Fig. 1 and the relevant data are given in Table 1.

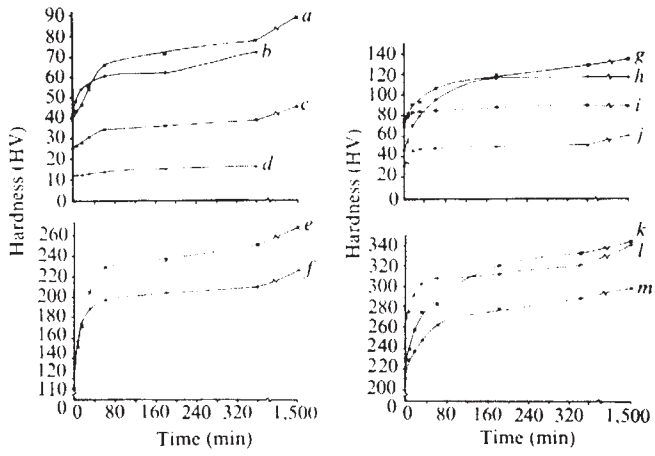


Fig. 1 The effect of ultrasonic agitation on the hardness of immersed metals. Metals with the same crystallographic structure are grouped together wherever possible: *a*, Mg; *b*, Zn; *c*, Cd; *d*, Sn; *e*, mild steel; *f*, Ni; *g*, brass; *h*, Cu; *i*, Nb; *j*, Al; *k*, Zr; *l*, Ti; *m*, Ta.

Table 1 Changes in hardness of immersed metals produced by ultrasound and during subsequent storage

Metal	Crystal structure	Hardness HV			Hardness changes (%)		Storage temperature (K) / Melting point (K)
		Initial	After 1,500 min agitation	After 1 month storage	After 1,500 min agitation	After 1 month storage	
Aluminium	f.c.c.	32†	61	43	+ 91	−29	0.31
Copper	f.c.c.	47†	117	93	+149	−21	0.22
Nickel	f.c.c.	110†	225	190	+105	−16	0.17
Brass 70/30	f.c.c.	75†	134	113	+ 79	−16	0.23
Stainless Steel 18/8	f.c.c.	249‡	450	294	+ 81	−35	0.17
Niobium	b.c.c.	70†	89	75	+ 27	−16	0.11
Tantalum	b.c.c.	210‡	299	276	+ 42	− 8	0.09
Mild steel	b.c.c.	142†	251	169	+ 77	−33	0.16
Cadmium	c.p.h.	25.5*	45	39	+ 76	−13	0.49
Magnesium	c.p.h.	40*	92	69	+130	−25	0.32
Zinc	c.p.h.	41†	72§		+ 76		
Zirconium	c.p.h.	227‡	345	281	+ 52	−16	0.14
Titanium	c.p.h.	270‡	342	274	+ 27	−20	0.15
Tin	Tetragonal	11.8*	16.0§		+ 36		

f.c.c., face-centred cubic; b.c.c., body-centred cubic; c.p.h., close-packed hexagonal. Microhardness load: *25 g; †50 g; ‡100 g.
§After 360 min.

The increase in hardness produced by ultrasound was not uniform but varied considerably over the surface; this was particularly noticeable on some metals which perforated during bombardment. Variations were also observed when the microhardness was measured at different areas on the surface, and because of this the readings were always taken on the same central area of the specimen. These variations may result from the presence of a standing wave which would form nodes and antinodes in the water above the probe.

The hardness of all the metals except tin, which showed only a slight change, increased rapidly at first and then tended to reach a steady value; a further increase in hardness was usually observed over the period of bombardment 360–1,500 min (Fig. 1).

There are certain similarities in the patterns of the percentage increase in hardness of metals which have the same crystallographic structure (Fig. 1). Face-centred cubic metals increased by about 80-150%, whereas body-centred cubic metals showed a much lower change of 27–77% and the change in close-packed hexagonal metals ranged from 27–130%. Tantalum, which is much purer than niobium showed less resistance to dislocation movement and underwent a higher increase in the percentage hardness. The lattice of brass was more strained than that of copper, which resulted in a much lower increase.

The increase in hardness of a metal exposed to ultrasound may result from the cavitation that occurs at the metal surface. The pressure in cavitation bubbles before they collapse has been calculated to be several thousand atmospheres[4] and, as the cavities implode, shock waves deform and harden the surface. Thus the lattice of the material at the surface of the metal may be distorted, and internal strains may be present in the surface layers.

A very significant decrease in the hardness during storage was observed with many of the metals, and in some cases, such as Ti, the value dropped to almost the initial reading. This softening process is too high to be a result of annealing at room temperature but could be caused by recovery at room temperature. There is a definite trend in metals with the same crystal structure (Table 1) between the percentage decrease and the ratio of storage temperature to melting point. Greater percentage decreases occur with the higher ratios which suggests that it is the recovery of point defects which gives the observed softening.

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Anion coordination geometry as a determining factor in crystallographic shear

ATTENTION has been drawn to some of the consequences of anion polarisation in one crystallographic shear (CS) system, the reduced titanium oxides[1]; particularly to the effect on crystal energy and cation coordination geometry. The existence of polarisation in typical CS structures is indicated by the values of certain common properties—the dielectric constant and refractive index are both high—by the more direct, experimental determination of net ion charges in some double CS (column, or so-called 'block') structures[2], and by the characteristic persistence of the cation coordination geometry[3], which is invariably octahedral.

Polarisation produces some directed covalency. The implications of this for anion coordination geometry are immediate and simple, but far reaching. For a second row element of the periodic classification, such as oxygen, the maximum coordination number for covalent bonding is CN(O) = 4. Thus, in ordered, reduced-rutile structures there can be only one set of CS planes: this raises the coordination number of anions at the CS planes from CN(O) = 3 in the rutile parent, to the maximum CN(O) = 4. On the other hand, when an ReO_3-type structure is reduced by CS, CN(O) is increased from 2 to 3 for one set of CS planes (slab structures). A second, intersecting set of CS

planes is possible before the maximum CN(O) = 4 is attained, thus producing column structures. True block structures—with three intersecting sets of CS planes—could not exist, because CN(O) = 5 would be produced (as it would in column structures derived from a rutile type parent).

Because CN(O) $\leqslant$ 4, the minimum stoichiometry for any CS structure MO_x will be x_{min} = 1.0, 1.5, 2.0 for cation coordination numbers CN(M) = 4, 6, 8, respectively. (This considers only the CN(M) values appropriate to the common regular cation coordination polyhedra: tetrahedron, octahedron and cube.) The stoichiometry of the parent structure must be $x > x_{min}$. Therefore, for octahedrally coordinated cations, the most reduced CS derivative will have $x = x_{min} = 1.5$ (compare with corundum type M_2O_3). On the other hand, CS in reduced fluorite type oxides is immediately ruled out, even though their bonding is of a covalent character[4]; because already, in the parent structure, CN(O) = 4, and $x = x_{min} = 2.0$.

These deductions are all in complete accord with a vast amount of experimental evidence. Furthermore, the 'distortions' from conventionally idealised structures (with regular $[MO_6]$ octahedra) to the real structures, which occur in the parents and in CS, column and bronze type derivatives, clearly show the strong tendency towards anion coordination symmetries appropriate to sp, sp^2 and sp^3 hybridisation, and characteristic of CN(O) = 2, 3 and 4.

For tetrahedrally coordinated cations the situation is unclear: no transition metal oxide is known to retain exclusively CN(M) = 4 on reduction. For example, although CrO_3 contains only $[CrO_4]$ tetrahedral groups, the (high pressure) structure of Cr_6O_{15} contains $[CrO_6]$ octahedra in addition to $[CrO_4]$ tetrahedra, whereas the (ambient pressure) structures of the reduced β and γ oxides are unknown[5].

In other metal oxide systems either the cations are not of variable valency (which is essential for CS in a binary system) or the situation is complicated by the effects of stereochemically active lone pairs of electrons (Ge, Sn, Pb, Sb, Bi)[6]. Perhaps they have been insufficiently studied. That is certainly true of systems with other anions, such as fluoride. There, however, polarisation effects will be much smaller than with the oxide ion, and there is certainly no evidence so far of CS in metal fluorides, although it does occur in oxide fluorides.

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Molecular mechanism for missense suppression in *E. coli*

The missense mutation *trpA36* results in a Gly (GGA) → Arg (AGA) amino acid substitution at position 211 of the tryptophan synthetase A protein[1]. Suppressors of *trpA36* have been derived by mutation in *glyT*, the gene specifying $tRNA^{Gly\,2}_{GGA/G}$, and in *glyU*, which specifies $tRNA^{Gly\,1}_{GGG}$ (refs 2–4). Transfer RNA from strains carrying either of the *glyTsuA36* or *glyUsuA36* alleles inserts glycine into polypeptides, *in vitro* and *in vivo*, in response to the arginine codon, AGA[3,5,6]. We report here a comparison of the nucleotide sequences of $tRNA^{Gly\,2}_{GGA/G}$ and $tRNA^{Gly\,2}_{suA36(HA)}$; the *glyTsuA36(HA)* mutation results in a C→U base change at the 3′-end of the anticodon of $tRNA^{Gly\,2}_{GGA/G}$, which causes a subsequent, enzyme-catalysed modification of the A directly adjacent to the 3′-end of the anticodon.

^{32}P-labelled $tRNA^{Gly\,2}_{GGA/G}$, obtained from *glyT*$^+$ *E. coli* strains, was purified by the methods of Gillam *et al.*[7,8]. ^{32}P-labelled $tRNA^{Gly\,2}_{suA36(HA)}$ was obtained by infection of strain BF266 with the phage, λh80d*glyTsuA36*, (ref. 9) and was purified by BD-cellulose[7] and RPC-5 reverse phase chromatography[10]. The tRNAs were sequenced by the methods of Sanger and co-workers, as described by Barrell[11]. A complete account of the purifications and sequence procedures will be presented elsewhere.

Ribonuclease T_1 fingerprints of the two tRNA species are quite similar, differing only in the position, and sequence, of oligonucleotide T-13 (Fig. 1), which forms the anticodon loop of the tRNA molecule (Fig. 2). Oligo T-13, from $tRNA^{Gly\,2}_{GGA/G}$, has the sequence CCUUCCAAG; oligo T-13′, from $tRNA^{Gly\,2}_{suA36(HA)}$, has the sequence CCUUCUÂAG. A portion of both species has an unidentified modified U in the wobble position of the anticodon (Fig. 2). The *glyTsuA36(HA)* mutation, therefore, replaces the C in the third position (3′-

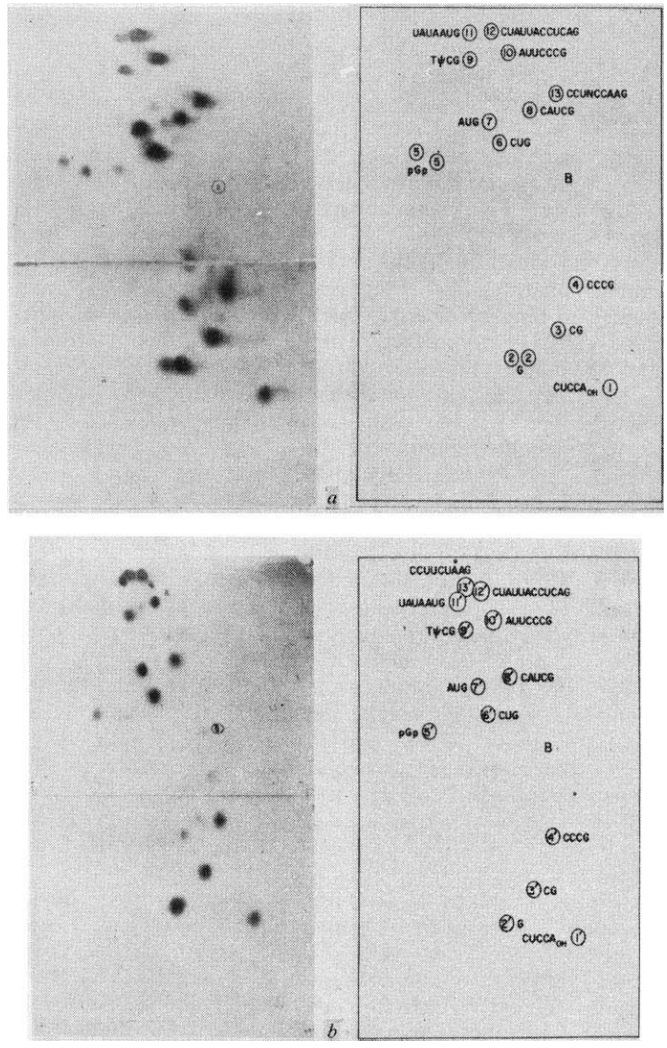


Fig. 1 A comparison of ribonuclease T_1 fingerprints of: (*a*) $tRNA^{Gly\,2}_{GGA/G}$; (*b*) $tRNA^{Gly\,2}_{suA36(HA)}$. First dimension electrophoresis on cellulose acetate at *p*H 3.5 was from right to left. Second dimension electrophoresis on DEAE paper in 7% formic acid was from top to bottom. The symbol B denotes the position of the blue dye marker (xylene cyanole FF).

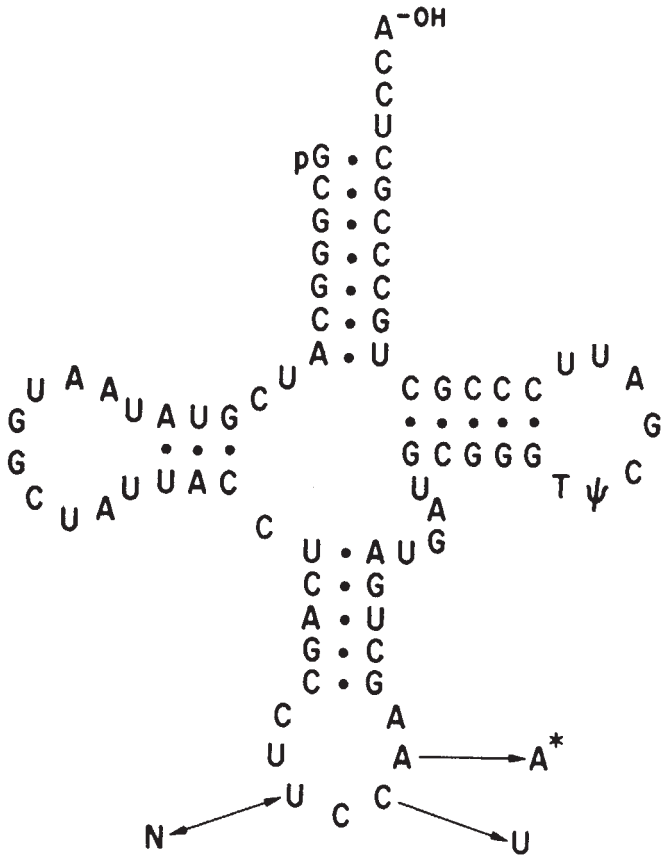


Fig. 2 $tRNA^{Gly\,2}_{GGA/G}$ drawn in the cloverleaf configuration. The arrows indicate the changes resulting from the *glyTsuA36 (HA)* mutation. N, an unidentified derivative of U, is present in a portion of both species. Å, as yet unidentified, is apparently a derivative of N⁶–carbamoylthreonyl–A.

end) of the anticodon of $tRNA^{Gly\,2}_{GGA/G}$ with a U, changing the anticodon sequence from UCC to UCU. The observed anticodon sequences are, as might be expected, complementary to the codons recognised by the tRNA species *in vitro* and *in vivo*.

Other missense suppressors can arise by alteration of the anticodons of, and thus the codons recognised by, particular tRNA species. For example, $tRNA^{Gly\,3}_{suA78}$, which inserts glycine into polypeptides in response to the cysteine codons, UGU and UGC, was derived from $tRNA^{Gly\,3}_{GGU/C}$ by a C→A base change at the 3′-end of the anticodon[12]. $tRNA^{Gly\,3}_{ins}$, which recognises the glycine codons GGA and GGG, was derived from $tRNA^{Gly\,3}_{GGU/C}$ by a G→U base change in the wobble position of the anticodon[13]. $tRNA^{Gly\,1}_{SupT}$, which recognises the glutamic acid codon, GAG, was derived by a C→U base change in the middle position of the anticodon of $tRNA^{Gly\,1}_{GGG}$ (ref. 14). In all cases, the anticodons of the suppressor tRNAs are complementary to the missense codons suppressed by these tRNAs.

As shown in Figs 2 and 3, the A adjacent to the anticodon of $tRNA^{Gly\,2}_{suA36(HA)}$ is modified, while the corresponding A in $tRNA^{Gly\,2}_{GGA/G}$ is not. The modified nucleotide, as yet unidentified, is converted to Ap by treatment with alkali; it exhibits an electrophoretic mobility at *p*H 3.5 (relative to Up) of 0.98, and chromatographic mobilities in systems A and B (ref. 11) (relative to Up) of 0.66 and 0.5 respectively. Since derivatives of N-[9-(β-D-ribofuranosyl)-purin-6-ylcarbamoyl]threonine (t^6A) are found adjacent to the anticodons of tRNA species recognising codons beginning with A (ref. 15), we suggest that the modified A adjacent to the anticodon of $tRNA^{Gly\,2}_{suA36(HA)}$ might be such a derivative. The enzymes responsible for this modification must recognise, at least in part, the anticodon sequences of prospective tRNA substrates. An analogous situation exists with $tRNA^{Gly\,3}_{suA78}$, which has 2–thiomethyl–6–isopentenyl-adenine (ms^2i^6A) adjacent to the anticodon; the corresponding A in $tRNA^{Gly\,3}_{GGU/C}$ is not modified (Fig. 3)[12]. Some suggestions have been made concerning the physiological significance of such modifications; for example, $tRNA^{Tyr}_{suIII}$ lacking the isopentenyl modification adjacent to the anticodon shows a greatly reduced ability to recognise the codon, UAG, *in vitro*, but appears to function normally in other respects[16].

The following observation suggests that the greatly reduced rate of aminoacylation of $tRNA^{Gly\,2}_{suA36(HA)}$ (10^{-4} times that of $tRNA^{Gly\,2}_{GGA/G}$ under identical conditions)[2,3,17] is a result of the base change in the anticodon, rather than the modification of the adjacent A. A tRNA capable of inserting glycine into polypeptides, *in vitro*, in response to the arginine codon, AGA, has been derived from $tRNA^{Gly\,2}_{GGA/G}$ by nitrous acid deamination, presumably of the C at the 3′-end of the anticodon. This altered $tRNA^{Gly\,2}$, chromatographically different from $tRNA^{Gly\,2}_{suA36(HA)}$ and probably lacking modification of the A adjacent to the anticodon, exhibits a greatly reduced rate of aminoacylation[17]. The role of the anticodon sequence in the aminoacylation reaction has been suggested for other tRNA species. If the anticodon of $tRNA^{Trp}_{UGG}$ is changed from CCA to CUA, the altered tRNA can be enzymatically aminoacylated with glutamine[18]. If a C in the anticodon of $tRNA^{Met}_{f}$ or $tRNA^{Val}$ is chemically modified to U, the tRNA can no longer be enzymatically aminoacylated[19,20]. Preliminary attempts to isolate unmodified $tRNA^{Gly\,2}_{suA36(HA)}$, in order to test this hypothesis directly, have been unsuccessful; in fact, modification of the A adjacent to the anticodon has been found in the uncleaved precursor of $tRNA^{Gly\,2}_{suA36(HA)}$ (S. Chang, and J. C., unpublished results).

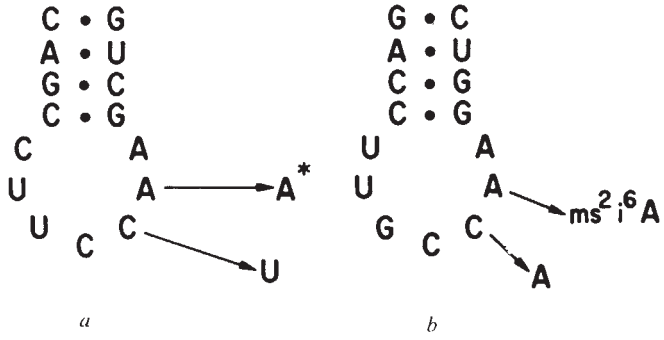


Fig. 3 A comparison of the anticodon changes and resulting modificational changes of: (*a*) $tRNA^{Gly\,2}_{GGA/G} \rightarrow tRNA^{Gly\,2}_{suA36(HA)}$, and (*b*) $tRNA^{Gly\,3}_{GGU/C} \rightarrow tRNA^{Gly\,3}_{suA78}$. In each case, substitution of the base at the 3′-end of the anticodon results in a modification of the adjacent A.

GlyTsuA36 mutations cause pleiotropic effects, of varying severity, on the cell; these effects are due, at least in part, to the loss of GGA translating ability, since they are reversed by the presence of *glyT*⁺ (normal $tRNA^{Gly\,2}_{GGA/G}$) or *glyVins* ($tRNA^{Gly\,3}_{GGU/C} \rightarrow tRNA^{Gly\,3}_{GGA/G}$) (refs 2, 4 and 13). For example, the *glyTsuA36(HA)* allele is semilethal when haploid[4]. The *glyTsuA36(159)* allele, which is probably the result of two or more closely linked mutational events, renders the cell tryptone-sensitive; when shifted from minimal to complex medium, cells carrying the *glyTsuA36(159)* allele cease normal cell division, form long aseptate filaments, and die[2,21]. Nucleotide sequence studies on $tRNA^{Gly\,2}_{suA36(159)}$ may suggest a molecular basis for the less severe pleiotropic effects of *glyTsuA36(159)*; *glyTsuA36(159)* may specify a $tRNA^{Gly\,2}$ with the *glyTsuA36 (HA)* anticodon alteration plus a second nucleotide substitution which allows the tRNA to recognise with low efficiency

the glycine codon, GGA, in addition to the arginine codons AGA and AGG (ref. 4).

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X-ray diffraction studies of DNA at reduced water contents

REDUCED water contents probably stimulate most closely the local environment about DNA when it is complexed with protein. At low water contents, less than about six water molecules per nucleotide, the equilibrium conformation for pure DNA is the A form[1,2]. The A form does not alter with base composition[3] whereas the B states of DNA are strongly correlated with chemical composition[4]. The A conformation (A state) is the only one which can be called a distinct form. There are many B type conformations all having ten base pairs per turn. 'C form' is a misnomer. We use the term 'C state' or type for structures having a pitch between 29 and 33A and a diffraction intensity distribution of weak, strong, then weak on the first three layer lines.

Since the discovery of the A form[1], all experiments on A-DNA where lattice formation could be detected, have shown the presence of a crystalline state. We have specifically looked for noncrystalline A-DNA but have not found it. In fibres, one might argue that orientation would favour and highlight crystalline regions, but this would not be the case for unoriented gels. We have dried wet gels to about 70% DNA concentration and subsequent X-ray scattering experiments have shown only B or crystalline A patterns.

As it has been reported that the circular dichroism and Raman spectra of DNA in 80% ethanol is the same as A-DNA in films and fibres[5,6], we decided to study the conformation of DNA in this solvent.

Indeed, we found that DNA did adopt the A conformation in 80% ethanol and that this A state was always crystalline and quite similar to that previously observed in gels and fibres (see Fig. 1). We also found that, after the addition of ethanol to a DNA gel, complete formation of the A state could require up to several hours and that, upon the B to A transition, spontaneous preferential orientation sometimes occurred.

In the nucleus, conditions for DNA crystallisation are obviously very unfavourable because of the protein–DNA and water–DNA linkages which are stronger than DNA–DNA interactions. Consequently, it is of biological interest to examine the dehydration of DNA under conditions where A transitions are inhibited.

At high water contents in solution and in fibres DNA assumes conformations of the B type[2,7] and when a B-DNA gel or fibre is slowly dried at lower relative humidity (r.h.), a transition to the A form is nearly always observed. But, if a hydrated fibre is maintained under tension while it is dried rapidly, the 30% contraction in length required for a B to A transition is inhibited and other conformations are adopted. When a wet gel of *Clostridium perfringens* DNA (69% A-T) is maintained under tension while drying at 20°–25° C, subsequent X-ray fibre patterns show diffraction of the P type at NaCl contents less than 2% and of the

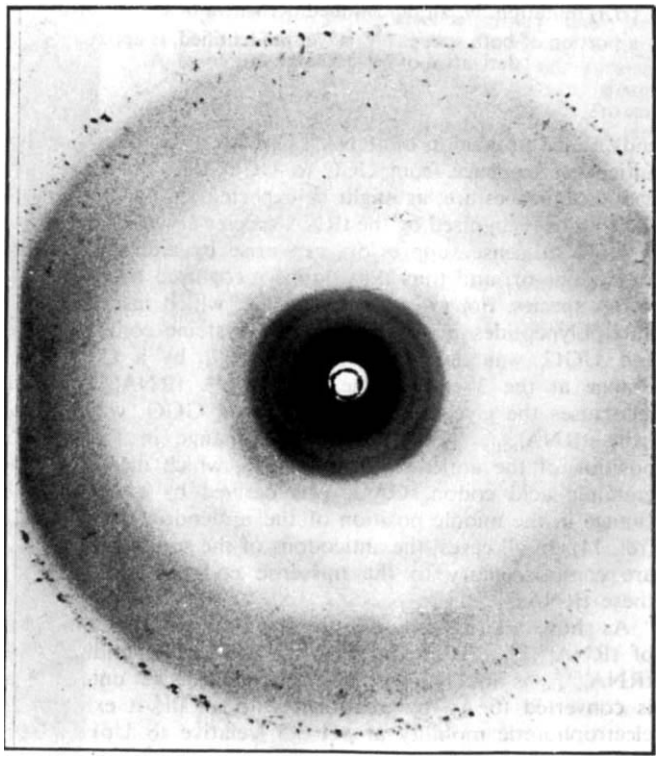


Fig. 1 A pattern of calf thymus DNA in 80% ethanol taken with a torroidal camera in air in a glass capillary tube. The concentration of DNA was about 5% but it was present as a precipitate. The diffraction is that of the crystalline A state and preferential orientation was observed perpendicular to the length of the capillary. The ring at 3.14 Å is from silicon powder on the capillary. Similar A patterns have been observed with *Cl. perfringens* DNA in 80% ethanol.

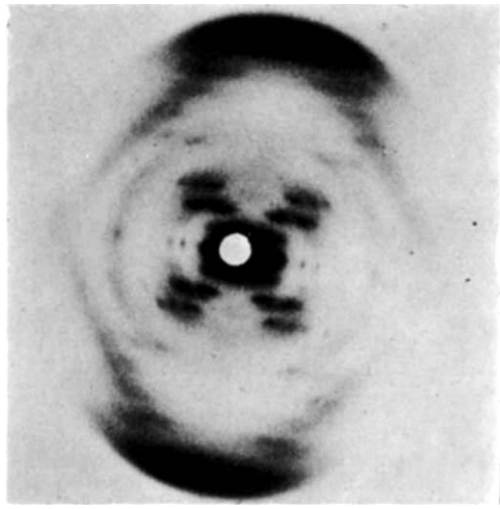


Fig. 2 A crystalline B pattern from *Cl. perfringens* DNA at 44% r.h. showing a small amount of A form (notice the slight splitting of the second layer line). The fibre which contained 5% excess salt was dried on a fixed platinium support at 37° C.

T type at higher salt concentrations. (The nature of these patterns has been described previously[8]; in short both the T and P structures are more highly wound and have smaller radii than the A, B or C structures.) The P and T states are stable for at least several days at 66% r.h. even if the fibre is freely suspended. But if the r.h. is raised to 98% and then returned slowly to 66% r.h., an A diagram appears. (Similar hysteresis effects have also been observed by Dr. A. M. Levelut at the Laboratoire de Physique des Solides, Orsay.)

If a gel of *Cl. perfringens* DNA on a fixed support is quickly dehydrated by drying at 37° C at r.h. < 66%, a B state is often obtained. These X-ray patterns are more crystalline than any other sodium B patterns yet observed. Some patterns (as in Fig. 2), however, show the presence of small amounts of A-DNA implying the existence of a metastable mixure. This low humidity B state is stable for at least 2 d at 44% r.h. The lithium salt of his DNA gives a crystalline B pattern at lower r.h. (ref. 8) but no C pattern has been observed with the lithium salt. Experiments with the magnesium salt of *Cl. perfringens* DNA (Mg-DNA) give a pattern at lower humidities which resembles those of C states in the intensity distribution on the lower layer lines but in which the ninth layer line, which has been related to a slight base tilting, is much weaker than in the C patterns. Thus *Cl. perfringens* DNA can adopt at least five different forms at the same water content.

It was accidentally observed that the sodium salt of calf thymus DNA (Na-DNA) gave a C pattern (S.B. and P. Tougard, unpublished) which was almost identical to the published lithium salt C diagrams (see Fig. 3). Subsequent experiments with a variety of pulling conditions and salt contents failed to give C patterns. It was then found, however, that calf thymus DNA dried at 37° C (between 30 and 66% r.h.) usually gives a C pattern. This state is metastable at 66% r.h. and 'decays' into the A form after about a day. An immediate C to A transition occurs at lower r.h. if a C-DNA fibre is cut loose at one end from its support. This rapid transformation is accompanied by a 30% contraction in fibre length. Calf thymus DNA can also give very crystalline B patterns at 30-66% r.h. when dried at 37° C. For calf thymus DNA, it seems that high salt conditions favour the metastable B state, whereas at salt contents less than about 3%, C structures seem to predominate.

Stable states are produced by several agents which are known to block the B to A transitions. The result is that at lower humidity new conformations are adopted, all of which are more highly wound than the A state. One agent known to block B to A transition is the lithium ion. The A form has never been observed with calf thymus Li-DNA (nor with *Micrococcus luteus or Cl. perfringens* DNA in this laboratory). Instead a C state is found at lower r.h. (ref. 10). Calf thymus Mg-DNA also does not exist in the A form but instead adopts a C configuration at lower r.h. (ref. 11); and in mixtures of sodium and magnesium salts, the relative amount of A-DNA we observed was far less than the Na^+/Mg^{2+} ratio. When glucose residues are present, as in phage T2 DNA, or with nonintercalating acridine orange or proflavin, instead of the A form a structure having eight or nine base pairs per turn exists at r.h. less than 80% (refs 12, 13). In fact, the fibre patterns shown by T2 DNA at low r.h. (ref. 12) are very similar to those of our P form. In chromatin the A form has never been detected and experiments in this laboratory show that small amounts of histone protein also inhibit the B to A transition in fibres. Thus, even if the requirement for crystallinity were not sufficient to prevent an A transition *in vivo*, these blocking agents, some of which are quite common in the cell, would strongly inhibit it.

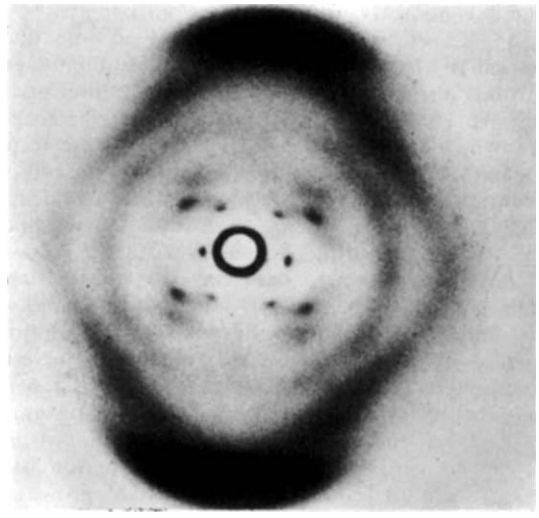


Fig. 3 A sodium C pattern from calf thymus DNA at 44% r.h. having a layer line separation of 31 Å. The fibre was dried on a fixed support at 37° C and over 44% r.h.

On the other hand, A-DNA, the only state which is invariant with base composition, might provide the best substrate for both RNA and DNA polymerases. Yet our results strongly imply that the A form of DNA can exist *in vivo* only when hybridised with RNA, for here DNA is forced to adopt the A configuration[14]. It may be that RNA or ribonucleotides play the role of a catalyst in transcription and replication by driving DNA to the A-state.

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RNA synthesis specific for an integrated adenovirus genome during the cell cycle

WHEN adenovirus transforms a cultured mammalian cell, substantial portions of the viral genome can be integrated into the host cell chromosomes and at least a fraction of this integrated genome is transcribed[1]. Approximately 50% of the early sequences observed in the lytic infections are synthesised in the transformed cell[2]. In randomly growing transformed rat embryo cells, the virus transcripts in the nucleus are in the large molecular weight, heterogeneous RNA covalently linked to cellular RNA[3]. These transcripts are apparently processed before they appear on cytoplasmic polyribosomes[3]. To date, there has been little evidence concerning the synthesis and post-transcriptional modifications of viral transcripts during the cell cycle in the transformed cells. The first question to be asked is, is there continuous synthesis of viral transcripts from at least some portion of the integrated genome, or is synthesis limited to one phase of the cell cycle? We have investigated this question in synchronised rat embryo cells transformed with adenovirus type 2 (Ad2-T)[4]. The synthesis of viral RNA transcripts was correlated with the life cycle of the cell which can be defined as consisting of a period of DNA synthesis (S phase) followed by a period of growth before mitosis (G_2), mitosis (M), and another growth period following division prior to DNA synthesis (G_1). Our data indicate that virus-specific RNA was transcribed throughout the cell cycle along with cellular RNA synthesis. Viral RNA transcription was restricted during mitosis coincident with the restriction of cellular RNA synthesis.

All experiments were performed with a cloned population[5] which demonstrated levels of T antigen and synthesis of virus-specific RNA at least equivalent to those of the uncloned population. Cells were maintained in spinner cultures in Eagle's MEM (without calcium)[6] enriched with 5% dialysed calf serum and 1% foetal bovine serum.

Cultures were synchronised in S phase by exposure to thymidine. Incorporation of ^{14}C-thymidine into acid-precipitable material began immediately after release from a double thymidine blockade (Fig. 1). Incorporation reached a maximum at about 3 h and then declined during the 4–6 h after synchronisation. Autoradiography revealed that 85% of the cells were synthesising DNA during the period 1–5 h following resuspension as opposed to 22% at 7–11 h. Approximately 90% of the culture passed through mitosis during a 4 h interval beginning at about 6 h after synchronisation. If the cells were released in the presence of colcemid, between 60 to 70% of cells accumulated in metaphase by about 10 h and an additional 20 to 30% seemed to be arrested in prophase.

Synthesis of cellular and viral RNA was compared in a

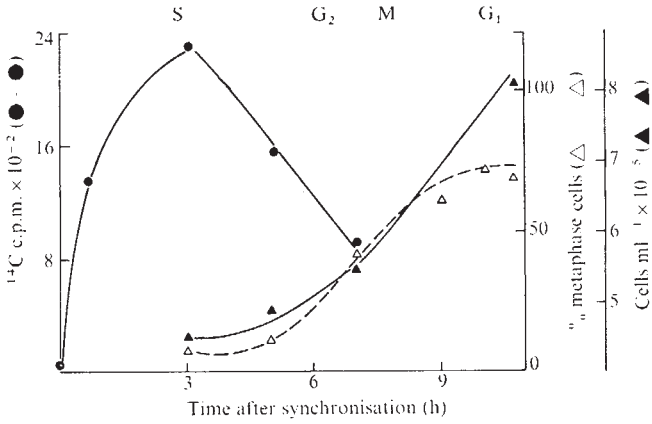


Fig. 1 Synchronisation of Ad2-T cells following thymidine blockade. A suspension culture of Ad2-T was synchronised with 2.5 mM thymidine by incubating the cells for 15 h in the presence of thymidine, resuspending in fresh medium for 9 h followed by an additional 14 h exposure to thymidine. At the indicated times after blockade 2 ml aliquots (approximately 10^6 cells) were transferred to tubes containing 0.5 μCi ^{14}C-thymidine (specific activity 40–60 mCi mmol^{-1}, New England Nuclear, Boston, Massachusetts). After incubation for 20 min at 37° C, the acid-insoluble radioactivity was determined as previously described[7]. Data were normalised to c.p.m. per 10^6 cells. At the time of resuspension an equal amount of the culture was treated with colcemid (0.025 μg ml^{-1}) and the accumulation in metaphase scored. If apparent prophase cells were counted along with metaphase cells, the final proportion of cells not in interphase was generally 85–90%. Cell concentration was determined with a haemocytometer and the mitotic index was determined with phase microscopy. ●, ^{14}C-thymidine; ▲, cells ml^{-1}; △, % metaphase.

population of cells progressing from S phase, through G_2 and into metaphase (Fig. 2). Aliquots of cells were exposed to ^{3}H-uridine for 1 h intervals during this period. RNA was prepared from whole cells[8] and analysed for total radioactive incorporation and for incorporation into virus-specific material using the low temperature DNA–RNA hybridisation procedure in the presence of 7.5 M urea[9]. Figure 2*a* illustrates that viral RNA synthesis corresponds to total cellular RNA synthesis. Incorporation of uridine into both viral and cellular RNA increased following release from thymidine, reached a maximum in S and steadily declined as the population proceeded through G_2 into metaphase-arrest.

The extent of restriction of viral RNA synthesis during mitosis was determined using metaphase-arrested cells collected from albumin density gradients as described in the legend of Table 1. Interphase cells, subjected to the same manipulative procedures, were added to the metaphase cells to form populations of defined content. These mixtures were incubated at 37° C with ^{3}H-uridine for 1 h and the total cellular RNA was prepared as described (Fig. 2). Incorporation into virus-specific RNA decreased linearly with increasing proportion of metaphase cells and approached zero. This decrease paralleled that of total cellular RNA synthesis suggesting that synthesis of both species is restricted to the same extent during metaphase.

Since the G_2 period is short and is not clearly defined following thymidine synchronisation (Fig. 1), it could be argued that viral RNA was synthesised primarily during S and that restriction during G_2 was masked by the entry of cells into mitosis. The nearly complete restriction of viral RNA synthesis during metaphase made a more precise examination of the G_2 phase possible. A culture was synchronised with thymidine, and colcemid was added at the time of release from the second blockade (Table 1). The culture was divided into three aliquots which were continuously exposed to ^{3}H-uridine for 6, 4, and 2 h before

collection of metaphase cells. The advantage of this approach is that by isolating metaphase cells, the incorporation during a given labelling period represents synthesis for a maximum of that time before mitosis. For example, cells incorporating radioactivity in the 2 h interval immediately preceding the collection of metaphase-arrested cells should represent synthesis by a fairly homogeneous population of cells in G_2. As expected metaphase cells collected from the population exposed to radioactivity for 6 and 4 h contained significant amounts of labelled virus-specific RNA. A lower level of incorporation was found in the third population labelled for 2 h. This latter result clearly indicates synthesis of viral RNA during G_2.

Between 6 and 10 h after thymidine synchronisation, the population of cells passed from G_2, through mitosis into G_1 (Fig. 1). No evidence of DNA synthesis was observed in this population until 9 h later. Aliquots of cells were exposed to ^{3}H-uridine for 1 h intervals beginning 8 h after synchronisation in S phase and RNA was prepared as previously described (Fig. 2*b*). There was minimal incorporation of uridine into both cellular and viral RNA coincident with the time of mitosis. Incorporation then rose to fairly constant levels as the population progressed into G_1. Viral RNA synthesis seems to follow the resumption of cellular synthesis following mitosis. There is a steady decay in synchrony as the population progresses from S phase to G_1. The following techniques to initiate synchrony in G_1 were surveyed but proved unsuccessful: selective detachment of mitotic cells[10], release from density growth inhibition[11], serum deprivation[12], and isoleucine deletion[13].

We conclude that at least some virus-specific RNA was synthesised at all times during the cell cycle except mitosis.

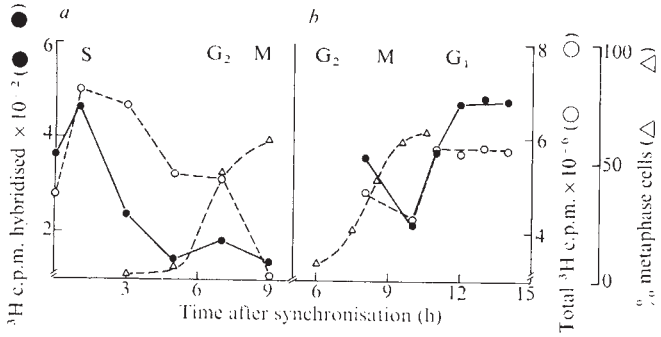
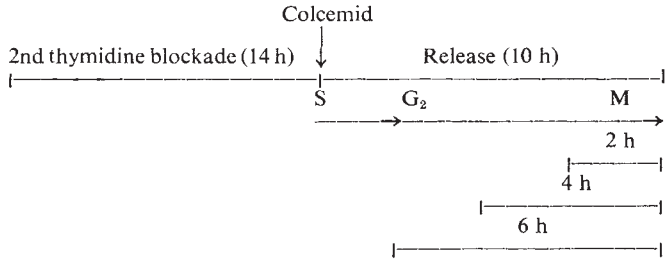


Fig. 2 Cellular and viral RNA synthesis following thymidine blockade. Ad2-T cells were used directly as S phase cells following thymidine synchronisation. At the indicated times 4 × 10^7 cells were suspended in 4.0 ml of complete medium at 37° C containing 200 μCi of ^{3}H-uridine (specific activity 720 Ci mmol^{-1}, New England Nuclear, Boston, Massachusetts). After incubation for 1 h, cells were washed, resuspended in STE (0.05 M Tris-HCl, *p*H 7.4; 0.001 M EDTA; 0.1 M NaCl) containing 1% sodium dodecyl sulphate (SDS) and 1% mercaptoethanol, and extracted three times at room temperature with phenol saturated with 20% STE. Nucleic acid was precipitated for 16 h at −20° C with 0.15 M NaCl and 2.5 volumes ethanol. The precipitate was suspended in 1.0 ml HSB (0.01 M Tris-HCl *p*H 7.4, 0.5 M NaCl and 0.05 M $MgCl_2$, digested with 100 μg DNase I at room temperature for 30 min, and the RNA reprecipitated. RNA was dissolved in 2 × SSC and the Cl_3CCOOH insoluble radioactivity and virus-specific radioactivity determined. DNA–RNA hybridisation was carried out in a solution of 2 × SSC containing 7.5 M urea, 10^{-2} M Tes (N-*tris* (hydroxymethyl) methyl-2-aminoethane sulphonic acid) buffer, *p*H 7.0, and 0.05% SDS. Equal aliquots of individual samples were incubated with nitrocellulose filters containing 8 μg of alkali denatured adenovirus 2 DNA and blank filters at 37° C for 4 d. Pancreatic RNase resistant radioactivity was determined. Virus-specific RNA was corrected for recovery from phenol extraction and for nonspecific binding using the amount of radioactivity bound to filters not containing adenovirus DNA. This represented 20–25% of the specific binding. *a*, Colcemid (0.025 μg ml^{-1}) was added at the beginning of S phase; *b*, colcemid was not added allowing the cells to pass through mitosis into G_1. ●, Virus-specific radioactivity; ○ total radioactivity; △, % metaphase cells.

Table 1 Extent of viral RNA synthesis in G_2

Interval before metaphase (h)	Cell cycle stage	Total c.p.m. × 10^7	Hybridisable c.p.m.
6	S + G_2	3.87	545
4	late S + G_2	1.62	228
2	G_2	0.77	178



The diagram below the table outlines the experiment as described in the text. RNA was extracted and assayed as described in Fig. 2 from metaphase arrested cells. At the end of each incubation period 4 × 10^7 cells with approximately 60–70% in metaphase arrest were mixed with bovine albumin resulting in a final concentration of 30%. Gradients were formed by successive addition of 1 ml each of the cell mixture, 23%, 20% and 17% albumin and 0.5 ml of 10% albumin at the top and were centrifuged in a Spinco SW65 rotor at 17,500 r.p.m. for 45 min at 5° C. 2 × 10^7 −3 × 10^7 cells with a metaphase index of 95–97% were recovered from the 17–10% interphase of two gradients.

During mitosis, the synthesis of viral RNA seemed to be restricted to the same extent as cellular RNA. These data permit the generalisation that the transformed state can be correlated with continuous functioning throughout interphase of at least some portions of the integrated genome. This result is in sharp contrast to that observed with H and L chain biosynthesis[14], induction of tyrosine amino transferase[15], and synthesis of histone mRNA[16], in cultured mammalian cells, none of which is continuous throughout interphase. The relative composition of the RNA transcripts synthesised in each phase must be explored. This analysis seems particularly important, since individual clones of Ad2-T have incorporated not only different portions of the adenovirus genome but also different proportional amounts of the same fragment (J. Sambrook, personal communication). Comparison of the RNA synthesised during the same phase of the cell cycle in different clones may provide information about the site of integration of the viral genome, especially if sufficient quantities of virus-specific RNA can be obtained for limited structural analysis. Of equal importance is the post-transcriptional processing in each phase. We are at present examining whether there is continuous export or selective exit of particular viral transcripts at unique times in the cell cycle. Further investigation with clones containing defined adenovirus segments, when synchronised, should provide useful information concerning both the maintenance of the transformed state and the expression of the host cell genome.

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Isolation of surface immunoglobulins from lymphocytes from chicken thymus and bursa

ALTHOUGH the chicken is more closely related to dinosaurs and crocodiles than to the mouse[1], the clear-cut demarcation between thymus-derived (T) and bursa-derived (B) lymphocytes in this bird[2,3] offers an excellent system for study of the two lymphocyte types. B cells have been demonstrated to possess surface immunoglobulin[3–5], while T cells have not, although it has been established that light chains at least are expressed on specifically activated chicken T cells[6]. So far, however, intact polypeptide chains have not been demonstrated on T and B cells. We now describe the isolation and partial characterisation of surface immunoglobulins of chicken B and T lymphocytes. We have evidence that both lymphocyte types have surface immunoglobulins consisting of light chains, μ chains and a heavy chain of molecular weight 40,000.

Lymphocyte suspensions were prepared from thymuses, bursas and spleens of normal chickens (outbred white leghorn–australorp hybrids; newly hatched or 3–4 months old) and thymuses and spleens of hormonally bursectomised[7] chickens (Table 1). The cell populations were greater than 95% viable and consisted predominantly of lymphocytes (bursa and thymus, approximately 95%; spleen approximately 80%). These suspensions were surface-radioiodinated as previously described (refs 8 and 9 and Table 1 and Figure legends); ^{125}I-labelled surface proteins were extracted by various methods as described in the legend to Table 1, and immunoglobulin was isolated by specific immunological precipitation using antiserum to chicken 7S immunoglobulin (IgY) which possessed activity directed against light chains and the γ (Y) heavy chain.

As Table 1 shows, ^{125}I counts were specifically precipitated from labelled surface protein preparations obtained from thymic, bursa and splenic lymphocytes. In one case where thymus cells of neonatal chickens (day 1) were extracted with 1% Nonidet P-40, 6 M urea (apparent *p*H 7.2) no significant ^{125}I counts were precipitated by antiserum to chicken immunoglobulin. Because the other thymus lymphocyte preparations contained specifically precipitable immunoglobulin, we believe that the negative result represents a technical problem which arises from the release of proteolytic enzymes during the lysis of whole cells with the detergent urea mixture (D. Haustein and J. J. M., unpublished observations). Between 1.5 and 3.5% of ^{125}I-iodide counts in high molecular weight cell surface protein

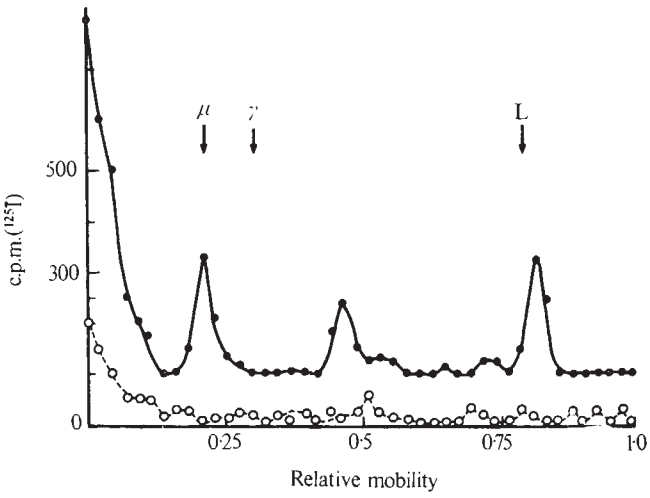


Fig. 1 Analysis by polyacrylamide gel electrophoresis in SDS-containing buffer of reduced ^{125}I-labelled surface immunoglobulins of chicken bursa lymphocytes. Sample is adult bursa, experiment I, Table 1. ●, Specifically precipitated immunoglobulin (RAFγG + GARG); ○, control precipitate (NRS + GARG). μ, γ and L indicate the positions at which human μ chain, γ chain and light chains migrated in this 9% gel.

of bursal lymphocytes were precipitated as immunoglobulin. Corresponding results for thymus lymphocyte populations varied from 1.0 to 2.4% under these conditions.

Although these results show that surface immunoglobulin can be isolated from chicken lymphocytes, they tell nothing about the class of such immunoglobulins because the antiserum used possessed activity for light chains. To investigate this problem we dissolved the precipitates in 3% sodium dodecyl sulphate (*p*H 6.8) containing 10% glycerol, 6M urea and 2% mercaptoethanol to cleave interchain disulphide bonds, and resolved heavy and light polypeptide chains by electrophoresis in 9% (w/v) polyacrylamide gels under conditions described by Laemmli[10]. Figures 1 and 2 illustrate the polypeptide chain distribution patterns for adult (3 months) bursal lymphocytes (Fig. 1) and neonatal thymus thymocytes (Fig. 2). These patterns are representative of all experiments. In both cases illustrated, components with mobilities characteristic of μ chains and light chains were resolved. Each pattern also contained a component with a relative mobility of approximately 0.5 which corresponded to a molecular weight of about 40,000 in terms of the standard proteins used. High molecular weight aggregate which did not enter the gel was also present in all chicken samples analysed. No definite components resembling the chicken γ (Y) chain (molecular weight 65,000; ref. 11) were found in any preparations.

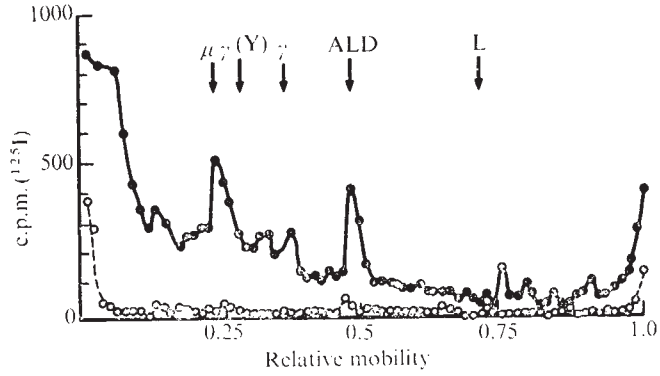


Fig 2 Analysis by polyacrylamide gel electrophoresis in SDS-containing buffer of reduced ^{125}I-labelled surface immunoglobulins on neonatal thymus lymphocytes (experiment IV, Table 1, turnover sample). ●, Specifically precipitated sample (FγG + RAFγG); ○, control, precipitate (MγG + RAMγG). μ, μ chain (chicken); γ (Y) chicken γ (Y) heavy chain; γ, human γ chain; ALD, aldolase; L, light chain (chicken). 9% polyacrylamide.

Table 1 Isolation of ^{125}I-labelled surface immunoglobulin from chicken lymphocytes

Experiment	Cell source	Extraction procedure	(^{125}I-iodide in precipitate) Specific system	Control system	Difference	% Initial counts (high mol. wt protein in sample) precipitated as Ig
I	Bursa (adult)	Turnover	12,600 ± 700 (*a*)	3,600 ± 100 (*b*)	9,600	1.5
II	Bursa (neonatal)	Turnover	15,000 ± 940 (*c*)	2,400 ± 90 (*d*)	12,600	3.5
III	Bursa (neonatal)	Turnover	9,600 ± 600 (*c*)	1,200 ± 50 (*d*)	8,400	3.5
		Acid-urea	4,800 ± 350 (*c*)	1,200 ± 80 (*d*)	3,600	3.2
IV	Thymus (neonatal)	Turnover	8,300 ± 700 (*c*)	2,800 ± 30 (*d*)	5,500	2.4
		Nonidet P-40 extract of pellet	11,700 ± 1,300 (*c*)	4,100 ± 300 (*d*)	7,600	0.6
V	Thymus (bursectomised)	Nonidet P-40 urea	1,800 ± 200 (*c*)	1,500 ± 80 (*d*)	N.S.	—
VI	Thymus (bursectomised)	Acid-urea	3,900 ± 300 (*c*)	900 ± 70 (*d*)	3,000	1.0
	Spleen (bursectomised)	Acid-urea	2,500 ± 130 (*c*)	300 ± 20 (*d*)	2,200	0.7
	Thymus (normal)	Acid-urea	5,900 ± 120 (*c*)	2,000 ± 40 (*d*)	3,900	1.3
	Spleen (normal)	Acid-urea	1,100 ± 40 (*c*)	400 ± 30 (*d*)	700	0.5
	Bursa (normal)	Acid-urea	12,600 ± 400 (*c*)	4,900 ± 130 (*d*)	7,700	2.5

Chicken lymphocytes were radioiodinated as follows. To 10^7 lymphocytes in 50 µl phosphate buffered saline (0.02 M phosphate; 0.15 M NaCl, *p*H 7.2) containing 20 µg lactoperoxidase (either prepared by the method of Morrison and Hultquist[23] or purchased from J. L. B. Laboratories, Granada Hills, California) were added 4 µl (approximately 300 µ Ci) carrier-free ^{125}I-iodide (Radiochemical Centre, Amersham). The reaction was initiated by addition of 10 µl of 0.03% H_2O_2 followed by mixing. The cells were incubated at 30° C for 5 min, after which the reaction was stopped by addition of large volumes of chilled phosphate buffered saline (PBS). Cells were washed twice at 4° C by centrifugation before lysis or metabolic release. Uptake of ^{125}I-iodide ranged from 10-20% of the initial amount. Between 5 and 10% of this cell-associated value was high molecular weight as judged by dialysis or trichloroacetic acid precipitation. Metabolic release (turnover) of surface immunoglobulins of labelled cells was achieved as before[24]. For extraction with Nonidet P-40 cells were incubated in 0.5% Nonidet P-40 in PBS at a concentration of 10^7 cells ml for 15 min at room temperature. Nuclei and insoluble particulate matter were removed by centrifugation. Acid-urea extraction was performed as described elsewhere[9]. In extraction with Nonidet P-40 urea, the cells were dissolved in 1% Nonidet P-40—6M urea; otherwise the conditions were as described above. All samples were dialysed against 0.15 M NaCl buffered to *p*H 8.0 with Tris-HCl before immunological analysis. Coprecipitated samples were washed three or four times. Data given here refer to ^{125}I counts (means ± s.e. of at least four replicates) associated with specific or control precipitates. Precipitation systems were (*a*) rabbit antiserum to chicken IgG (Y) immunoglobulin (contains specificity for light chains and γ (Y) chains) plus goat antiserum to rabbit IgG (Commonwealth Serum Laboratories, Melbourne); (*b*) normal rabbit serum plus goat antiserum to rabbit IgG; (*c*) carrier chicken Igµ (Y) plus rabbit antiserum to chicken Igµ (Y), and (*d*) mouse IgG plus rabbit antiserum to mouse IgG. All systems were calibrated to precipitate more than 90% of their homologous antigen. Data given represent counts from 2–5 × 10^6 lymphocytes.

A significant finding was the release of IgM by turnover from thymuses of newly hatched chickens. In these chickens the only existing immunoglobulin is maternal IgG (Y) released from the yolk, and there is no detectable IgM in the serum[12]. A second significant finding was that chicken bursa lymphocytes express surface immunoglobulin polypeptide light chains, µ chains and a component of molecular weight 40,000. It is not surprising to find light chains and µ chains because several workers using methods similar to ours isolated IgM immunoglobulin from human [13] and murine[14,15] B lymphocytes. The 40,000 molecular weight component was unexpected, but is consistent with the report that chicken B lymphocytes synthesise and apparently express on their surfaces a component of similar size which is antigenically related to µ chain[16] and termed H_o chain. A heavy chain of this size is interesting from an evolutionary viewpoint because the major immunoglobulin class in certain reptiles[17] and birds[18] contains a heavy chain of mass approximately 40,000 daltons. This heavy chain has been termed the nu chain[19] Although such a component is not readily detectable in chicken serum, it might constitute an important surface-associated receptor molecule in this species. We do not believe that this surface-associated heavy chain is a degradation product of µ chain because of the biosynthetic results of Choi and Good[16] and because the molecule has been obtained under conditions where we have not observed proteolysis in studies of mammalian lymphocyte surface immunoglobulin[20]. Ladoulis *et al.*[25] reported immunoglobulins of molecular weight 200,000 and 130,000 on the plasma membranes of rat thymus and spleen lymphocytes. The chicken surface immunoglobulins tended to aggregate and we could not estimate intact molecular weights. If they consist of L_2H_2 units analogous to surface immunoglobulins of man[13,15,20] and mouse[14,15,20], they should have molecular weights of about 185,000 and 125,000.

Our finding of immunoglobulins consisting of µ and light chains on chicken T cells is consistent with similar studies of mammalian T cells[15,20,21]. Biosynthesis has demonstrated immunoglobulin in thymocyte populations of normal chickens[22] and Theis and Thorbecke[6] inhibited specific delayed type hypersensitivity reactions in bursectomised chickens by antisera to immunoglobulin, concluding that chicken T lymphocytes have at least light chain antigenic determinants on their surfaces. We have now established that such cells also express µ chains and a chain similar to the H_o heavy chain described by Choi and Good[16]. Because our method gives a bulk average, it is not possible to decide whether µ chain and H_o chain occur on the same T or B lymphocytes.

The existence of IgM on the surface of mammalian T cells has been confirmed independently in several laboratories[21,25,26]. The presence of IgM on the thymocytes from newly hatched chickens which lack detectable circulating IgM, makes it improbable that

this immunoglobulin has been adsorbed from the serum.

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Effect of T cell depletion on the potentiated reagin response

IN the rat, infection with helminth parasites can have the remarkable effect of causing nonspecific potentiation of IgE (reaginic) antibody responses against antigens unrelated to those of the parasite. This phenomenon, which has been called the potentiated reagin response[1], has been found to encompass IgE responses to antigens as diverse as egg albumin, keyhole limpet haemocyanin and house dust[2] and has been produced with representatives of both nematode and trematode parasites[3].

The potentiated reagin response is but one manifestation of the potent IgE stimulating effect of helminths. Infections with these parasites also induce high levels of reaginic antibody specific for parasite derived antigens, and at least in man, cause greatly elevated levels of total serum IgE. Parasite-specific reagins which were first described in infections of the rat with *Nippostrongylus brasiliensis* and the rat and monkey with *Schistosoma mansoni*[4] have been demonstrated in various host–parasite systems in many species including man[5,6].

The potentiated reagin response provided a basis for experimental investigation of the mechanism of IgE stimulation in helminth infections. It is known that parasitic infection can only amplify an already existing IgE response: in the rat this must be induced before infection by inoculation of the antigen together with a conventional adjuvant[1,7]. IgE responses against different antigens may be simultaneously potentiated[2] while IgG responses remain largely unaffected[7,8]. The indications from these results are that live worms produce a factor which has the capacity to stimulate those immunocytes previously programmed for IgE production.

To find out whether this factor acts directly on IgE-producing B cells or whether its effect is produced through T cells, we examined the effect of T cell depletion and show here that this prevents potentiation of reagin responses in rats infected with the nematode *N. brasiliensis*.

Outbred female Hooded Lister rats (Animal Suppliers (London) Ltd), were used. Thymus deprived (B) rats were prepared by thymectomy at 4 weeks of age, followed by 850 rad whole body irradiation 2–4 weeks later. During

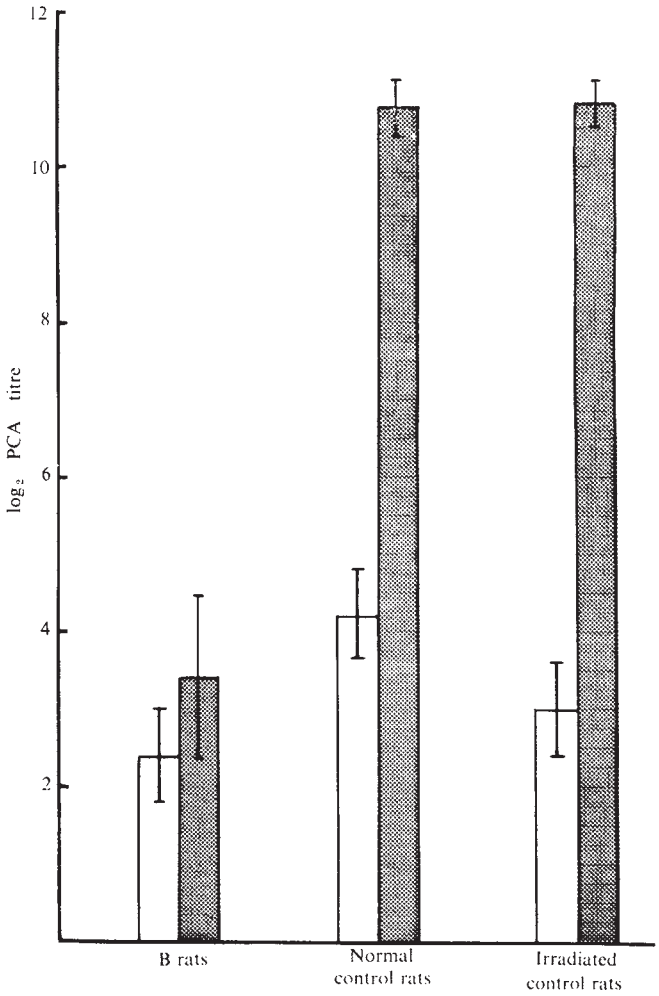


Fig. 1 Mean EA reaginic antibody levels 4 d before (□) and 14 d after (▨) *N. brasiliensis* infection. Eighteen B rats, 10 normal control rats and 8 irradiated control rats were immunised with 1 mg EA (58 d after thymectomy; 38 d after irradiation). Of these 9 B rats, 10 normal rats and 6 irradiated rats produced an EA IgE response and were infected with *N. brasiliensis* 28 d after immunisation. The means and standard errors shown above were calculated using logarithmically transformed antibody titres.

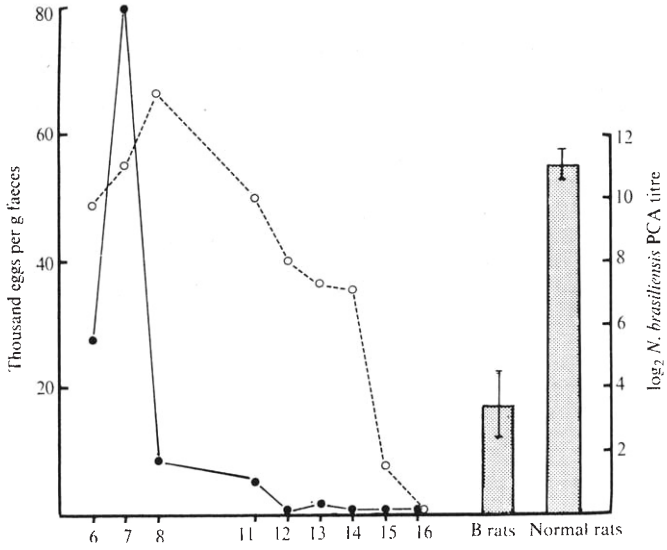


Fig. 2 Prolonged egg output in B rats (○) infected with *N. brasiliensis* compare with infected normal rats (●). Groups of 10 B rats and 5 normal rats were killed 19 d after infection when mean worm burdens in the small intestine were found to be 618 ± 255 (s.e.) for the B rats and 3 ± 2 (s.e.) for the normal controls. Of these animals 5 B rats and 5 normal rats had detectable circulating *N. brasiliensis* reagins and the mean levels (± s.e.) are shown above.

irradiation the distal parts of both hind limbs were shielded to allow auto-repopulation by bone marrow cells. This technique[9] has been shown to produce animals whose T deficiency resembles that of thymectomised animals prepared by irradiation and reconstitution with bone marrow cells. The animals were immunised 1–2 months later by intraperitoneal inoculation of 1 mg of either egg albumin (EA) (Sigma grade 5) or keyhole limpet haemocyanin (KLH) (Cal Biochem A grade) together with 10^{10} *Bortetella pertussis* organisms (Wellcome Biological Reagents). At appropriate times after immunisation, the animals were bled from the tail vein and IgE antibody levels were determined by titration of individual sera by the passive cutaneous anaphylaxis (PCA) technique[10]. The IgE-producing B rats together with IgE-producing normal rats were then infected by subcutaneous inoculation of 4,000 *N. brasiliensis* larvae and were bled for antibody estimations 12–14 d later when the potentiated reagin response normally reaches its peak.

Table 1 shows the results of one such experiment involving groups of B and normal rats immunised with either EA or KLH. It can be seen from the individual titres that whereas reagin responses were markedly potentiated in eight out of ten normal animals a significantly raised response occurred in only one of ten B rats. This latter animal is in fact the only B rat in which potentiation has occurred in four experiments.

Figure 1 shows the results of another similar experiment which included an additional control group of rats which had been irradiated without previous thymectomy. Again it is clear that the parasitic infection did not have any significant effect on the levels of EA reaginic antibody in B rats whereas the response of the normal animals was substantially raised. The results also show that the process of irradiation does not in itself interfere with the ability to form potentiated reagin responses. Two other experiments were performed which gave consistent results.

In these experiments we also studied some parameters of the immune response to the parasite itself since T-cell deficiency has been described as depressing immune responses in parasitic infections[11,12]. In normal adult rats,

Table 1 Effect of *N. brasiliensis** infection on egg albumin (EA) and keyhole limpet haemocyacin (KLH) IgE responses in normal and thymus deprived (B) rats.

	PCA titres 4 d before and 12 d after infection†			
	B rats		Normal rats	
	Before infection	After infection	Before infection	After infection
EA reagin responses	4	0	16	32,768
	4	16	16	1,024
	8	2	64	8,192
	16	16	8	64
	8	512	32	2,048
KLH reagin responses	16	64	8	64
	8	1	16	1,024
	8	2	8	2,048
	8	8	128	2,048
	8	16	4	1,024

The Hooded Lister strain of rats which we use normally produce a good IgE response on immunisation with doses of EA as low as 1 μg (ref. 18). Preliminary experiments with B rats showed that these did not have an IgE response following doses of 10 μg EA but that 33–50% did so following doeses of 1 mg EA. The ability to overcome effects of T cell deficiency by increasing the antigen dose has been reported in other systems[19,20]. In this experiment, 27 B rats were divided into groups of 15 and 13 and the animals of each group were immunised with 1 mg EA or KLH respectively (44 d after thymectomy, 30 d after irradiation). Groups of normal rats were similarly immunised. Five animals in each of the B groups developed a reaginic antibody response and they, together with a similar number of normal control animals, were infected with *N. brasiliensis* 26 d after immunisation.

* The method of culture of *N. brasiliensis* and preparation of the infective larval dose have been previously described[21,22].

† To estimate reaginic antibodies by the PCA technique 0.1 ml quantities of saline dilutions of the test sera were injected intradermally in Hooded Lister recipient rats, each injection being duplicated on a different animal. 48–72 h later the animals were injected intravenously with 2.5 mg of either EA or KLH together with 0.5 ml of 1% Evans blue which acts as an indicator of vasodilation. The skin reactions were examined after 20 min and the titres recorded above are the greatest dilutions of serum which gave reactions larger than 5 mm in diameter.

primary infection with *N. brasiliensis* is followed 10–12 d later by an immunological expulsion of worms from the small intestine. This so called 'self-cure' reaction proceeds exponentially over several days and is preceded by a fall in egg production by the parasite. Reaginic antibodies specific for *N. brasiliensis* appear during or shortly after worm expulsion and rise to reach high circulating levels. These manifestations of parasitic immunity were found to be markedly depressed in the B rats in our experiments. Egg production continued for a longer period than in the control animals, the expulsion rate of worms was decreased and the parasite specific IgE response was depressed or undetectable (Fig. 2).

Although it seems clear that T cells are involved in the potentiation of existing IgE responses by *N. brasiliensis* infection, their mode of action is still conjectural. Other workers using hapten-carrier immunisations have shown that carrier-specific T cells are heavily involved both as helpers[13–15] and suppressors[16,17] of B cells in anti-hapten IgE responses of rats and mice. In these situations, the T cell effect is antigen specific. The potentiated reagin response on the other hand is a nonspecific event and any theories about its mechanism must accommodate this element of nonspecificity as well as the requirement for T cells and the fact that it is only, or largely, IgE responses which are affected. T cells are known to produce a variety of soluble factors which can affect both cell-mediated and humoral immunity and it would seem most likely that it is through such factors that they exert their role in the potentiated reagin response.

Assuming that T cells exert a helper effect, potentiation could be brought about in one of two ways. In the first,

the parasite-derived potentiating factor activates a population of T cells to produce a substance which is capable of selectively stimulating IgE B cells regardless of their antigenic specificity. In the second, the parasitic factor activates T cells so that those which have preordained IgE helper functions (induced by previous immunisation) are driven to perform them. In the latter mechanism, the T cell effects on the B cell is antigen specific. It is equally possible that the potentiation of reagins is the result of release of B cells from the inhibiting effect of suppressor T cells but, in essence, the mechanisms outlined above would remain the same.

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Cortisol induction of glycerol phosphate dehydrogenase in a rat brain tumour cell line

HORMONAL regulation of enzyme levels in mammalian cells has been known for more than a decade[1] and the use of homogeneous populations of glucocorticoid target cells has advanced our understanding of the molecular mechanisms involved[2,3]. Using a specific antiserum we have now shown that cortisol induces glycerol phosphate dehydrogenase (GPDH, EC 1118) in a rat brain tumour cell line by increasing the number of GPDH molecules rather than by increasing the activity of each molecule. Also, the induction does not involve isozymes since GPDH from induced and uninduced cells is identical with respect to immunological titration, electrophoretic mobility and thermal sensitivity.

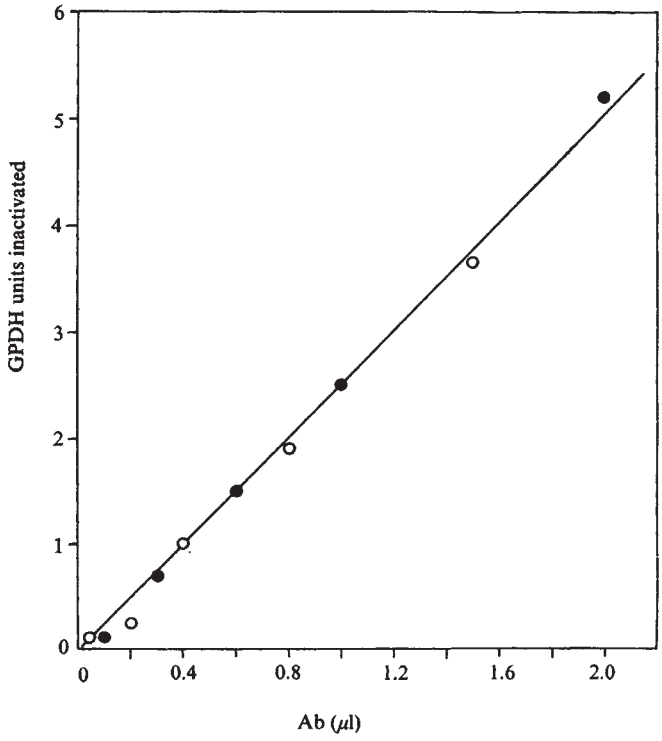


Fig. 1 Immunological titration of GPDH from control and cortisol-induced cells. Cells were grown in Ham's F-10 medium supplemented with 10% foetal calf serum, in Falcon T-250 plastic flasks as described previously[6,8]. Cortisol (when present) was added to a final concentration of 0.5 μg ml^{-1} for 3 d before collection. The cells were first washed twice with 3 ml volumes of 10 mM Na phosphate buffer, *p*H 7.5, containing 5 mM mercaptoethanol and 1 mM EDTA and then were collected in 1 ml of the same buffer using a rubber policeman to scrape the cells from the flask. After disruption by sonication cellular debris was removed by centrifugation for 1 h at 30,000 r.p.m. in a 30 rotor using a Beckman L2-65-B ultracentrifuge. The supernatants from induced and uninduced cells were assayed for GPDH activity as described previously[15] and the concentration of enzyme was equalised by concentrating the supernatant from uninduced cells using an Amicon ultrafiltration apparatus (PM30 membrane). Rabbit antiserum against purified rat brain GPDH was obtained as described previously[15]. The serum was initially diluted in 0.01 M phosphate buffer, *p*H 7.5 containing 1 mg of bovine serum albumin ml^{-1}, 1 mM EDTA and 5 mM mercaptoethanol and from this stock solution a series of dilutions of antiserum was made. A sample of 0.2 ml of each of the dilutions was added to tubes in triplicate. The high speed supernatant from induced and uninduced cells was used as the source of GPDH and a constant amount (0.1 ml, 200 U ml^{-1}) was added to each tube. After incubation at 37° C for 30 min an aliquot was removed and remaining GPDH activity was determined. Incubation overnight at 4° C did not result in further inactivation of GPDH and since the antibody–GPDH complex was enzymatically inactive it was not necessary to centrifuge the samples. The amount of enzyme remaining after incubation with antiserum was subtracted from that present in the abence of antiserum and the results were plotted as the number of units of enzyme removed against the microlitres of antiserum present. ○, GPDH from uninduced cells; ●, GPDH from cortisol induced cells.

The increase in the level of brain GPDH during the development of the rat is regulated specifically by cortisol[4]. This hormonal dependence has also been demonstrated in primary cultures of brain cells[5] and in RGC6 (C6) cells[6], a line of rat glial tumour cells[7]. The addition of cortisol or other glucocorticoids[6,8] to confluent C6 cells increases GPDH activity three-fold within 24 h and up to ten-fold over 5 d. This induction requires RNA synthesis, protein synthesis and the continual presence of cortisol[6,9].

Simple explanations for this increased activity—a direct effect of cortisol in the assay, increased overall protein synthesis and the presence of diffusible inhibitors or activators—have been ruled out[6]. We have investigated which of the alternative mechanisms is responsible for cortisol-dependent increase in GPDH in C6 cells. An increase in enzyme specific activity can occur if the catalytic activity of each molecule increases[10,11] or if the number of molecules increases[2,3,12,13]. These mechanisms are not mutually exclusive nor are they comprehensively explanatory since each could be achieved by various changes in the regulatory machinery of the cell.

Using rabbit antiserum directed against rat brain GPDH,

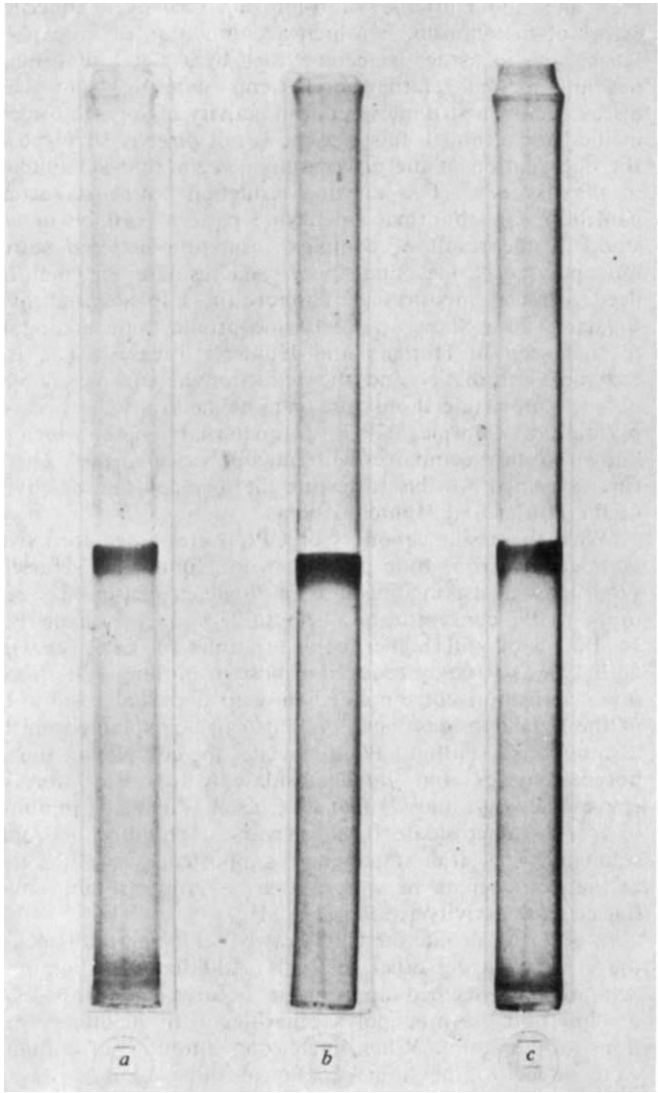


Fig. 2 Electrophoresis of GPDH from C6 cells (clone 2B-13). The cells were grown and collected as described in Fig 1. Electrophoresis was by the method of Davis[18] and the gels were stained for GPDH activity as described previously[15]. Each gel contained a total of 20 U of GPDH, where 1 U of activity is equal to the amount of enzyme which oxidises 1 nmol of NADH min^{-1} under the conditions of the assay. Supernatants were from induced cells (*a*), uninduced and induced cells (*b*) and uninduced cells (*c*).

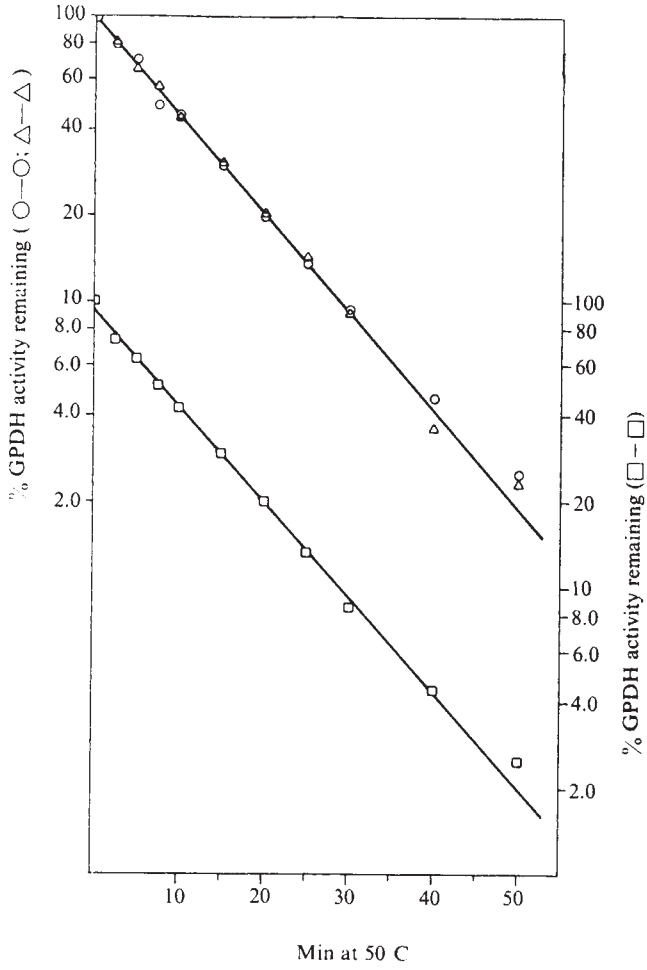


Fig. 3 Heat inactivation of GPDH from induced and uninduced cells. Cells were grown and collected as described in Fig. 1. Before concentration by ultrafiltration, the specific activities of GPDH in the high speed supernatants were 45 U mg^{-1} of protein and 188 U mg^{-1} of protein, for uninduced and induced cells respectively. The concentrations of GPDH at the zero time points (100% activity) were 207 U mg^{-1} for the induced (○), 206 U mg^{-1} for the uninduced (△) and 214 U ml^{-1} for the mixture (□) containing equal amounts of induced and uninduced enzyme activity. For clarity the data from the mixture are plotted using the ordinate on the right. Three sets of tubes (in duplicate) containing 200 μl of each of the enzyme solutions were incubated in a circulating water bath at 50.0±0.05° C for the indicated times. The tubes were sealed with parafilm to prevent evaporation. Immediately after removal from the bath, the samples were quickly cooled by swirling in an ice-salt slurry (−15° C) for 5 s and were then maintained at 0° C until completion of the experiment. Aliquots from each tube were assayed as described [15]

we have distinguished between the above mechanisms (Fig. 1) If the induction represents an increased number of molecules then equal amounts of antiserum will be needed to inactivate the same number of GPDH units from either induced or uninduced cells. If the induction is due to an increased activity per molecule then the amount of antiserum required to inactivate the same number of enzyme units from each source will be different. Figure 1 shows that equal amounts of enzyme are inactivated by equal amounts of antiserum. Therefore, the cortisol-dependent increase in GPDH activity in C6 cells represents an increase in the number of enzyme molecules.

The existence of more than one enzyme able to catalyse the same reaction has been known for some time. GPDH isozymes have been isolated from tissues of various species and at least two forms of GPDH have been demonstrated in mouse brain[14], while two distinct GPDH have been purified from rat brain[15]. In addition, the basal level of

GPDH in C6 clones[16] and in hybrids[17] is apparently regulated independently of the induced level of GPDH. Such data are consistent with the possibility that there is more than one structural gene for GPDH.

Since two electrophoretically distinct forms of GPDH have been purified from rat brain[15], we examined the mobility of GPDH by polyacrylamide gel electrophoresis as a test of the identity of GPDH in hormone-treated and untreated cells. Only one form of GPDH was found in induced (Fig. 2*a*) and uninduced (Fig. 2*c*) C6 cells. In addition, since only one band of enzyme activity was found when a mixture was run (Fig. 2*b*), we conclude that the GPDH in induced cells is electrophoretically identical to that in uninduced cells. Identity by polyacrylamide gel electrophoresis is not definitive proof that the basal enzyme is identical to the induced form, but shows that one of them does not represent an oligomeric form of the other and that there is no net charge difference between them.

Further confirmation of the identity of the induced and basal forms of GPDH was obtained from heat denaturation experiments. The ability of GPDH from control and treated cells to survive heating at 50° C (Fig. 3) showed that there was only one heat labile component in induced and uninduced cells. The single straight line obtained when supernatants from induced and uninduced cells are mixed and heated indicates that the GPDH from both sources is identical. These results, together with the electrophoretic and immunological data, argue against the possibility that the induction of GPDH activity in C6 cells involves the formation of an isozyme. The sensitivity of these procedures in detecting isozymes has been discussed[19] and more recently similar techniques were used to demonstrate the absence of isozymes in tyrosine aminotransferase induction in hepatoma cells[20].

We are now investigating the mechanism(s) by which the number of GPDH molecules increases. Obviously an increase in the rate of synthesis[12,13] of the enzyme or a decrease in its rate of degradation[21] or a combination of these could lead to an accumulation of GPDH molecules. The determination of the specific rates of synthesis and degradation will allow us to distinguish between these possibilities.

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In vitro restoration of deficient β-galactosidase activity in liver of patients with Hurler and Hunter disease

HUNTER'S and Hurler's syndromes are recessively inherited errors of metabolism, in which accumulation of mucopolysaccharides in tissues is accompanied by skeletal abnormalities and mental retardation. Patients suffering from these diseases show a strikingly reduced activity of β-galactosidase in the liver although this enzyme is not directly involved in the degradation of the glycosaminoglycans that accumulate in the tissues[1-3]. This enzyme reduction which is accompanied by an abnormal isoenzyme pattern[4] can be understood as the result of complex formation between stored mucopolysaccharides and the β-galactosidase enzyme. Indeed artificial mixtures of chondroitin sulphate and liver β-galactosidase show an isoenzyme profile quite analogous to that seen in Hurler's and Hunter's diseases[5]. The isoenzyme abnormality and the reduction in enzyme activity of such an artificial mixture can be neutralised by cetylpyridinium chloride (CPC), a quaternary base which is known to form complexes with mucopolysaccharides[6]. Therefore it seemed possible to restore the β-galactosidase activity of the Hurler and Hunter patients.

When increasing amounts of CPC were added to a liver homogenate from four patients with Hunter's or Hurler's syndromes a striking increase in β-galactosidase was seen, up to a CPC concentration of about 25 μg per mg liver (Fig. 1). But with still higher concentrations of CPC, enzyme activity was strongly reduced almost to nothing. The maximum activation corresponded only to a partial restoration of the β-galactosidase activity. This can be explained on the assumptions[6]: (1) that CPC dissociates the complex of mucopolysaccharides and β-galactosidase with restoration of enzyme activity; and (2) that CPC itself is a strong inhibitor of free β-galactosidase (that is, without bound chondroitin sulphate). The latter statement is supported by the fact that in the homogenate of a normal liver, strong inhibition of the enzyme activity is seen (Fig. 1).

In order to obviate the CPC drawback, I tried to dissociate the complex using other methods. Addition of albumin in acid medium seemed appropriate because of the possible precipitation of mucopolysaccharides with acidified solutions of albumin[7]. When increasing amounts of albumin were added to the homogenates of the patients' liver, β-galactosidase rose about five- to ten-fold, whereas in a normal liver an activation of only 10% was found. The restoration resulted in an essentially normal activity in the livers from two patients, while for the two other patients a final activity of about 40% of the mean normal value was achieved (Fig. 2). The highest restoration was found in the liver which had been stored for the shortest time.

It seems unlikely that therapeutic applications can result from the data presented here. If the albumin concentration

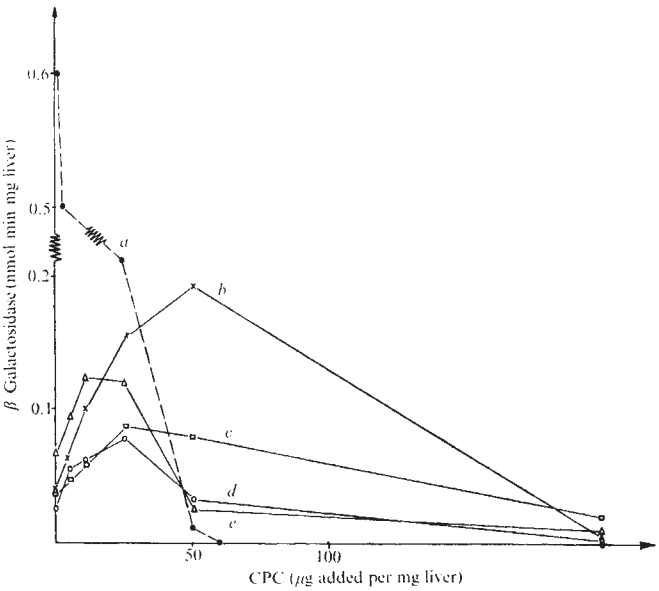


Fig. 1 Influence of increasing amounts of cetylpyridinium chloride on β-galactosidase activity in human liver homogenates of *a*, normal; *b*, Hunter; *c–e*, Hurler individuals. Autopsy material from patient *c* had been stored at −20° C for 4 yr while the three other liver pieces were stored for 7 yr. Liver homogenates (4% v/v) in distilled water were centrifuged after sonicating for 60 s and five cycles of freezing and thawing. Increasing amounts of CPC in distilled water were added to the supernatant of each individual and β-galactosidase activity was then measured as described[6].

of the *in vitro* experiments reflect the conditions for *in vivo* restoration, high intracellular concentrations of albumin are necessary. Moreover, the restoration of β-galactosidase activity will result in only a minor attack on the stored material. Only the accumulated galactose-containing glycolipids and gangliosides will be broken down, while the fundamental absence of α-iduronidase[8], sulpho-idurono-sulphatase[9], and N-acetyl-α-glucosaminidase[10,11] will not be corrected by the albumin treatment.

But, if the mental deficiency of patients with mucopolysaccharidosis is due to the neuronal accumulation of the glycolipids, *in vivo* restoration of β-galactosidase by infusion of large amounts of albumin early in life, might nevertheless be beneficial.

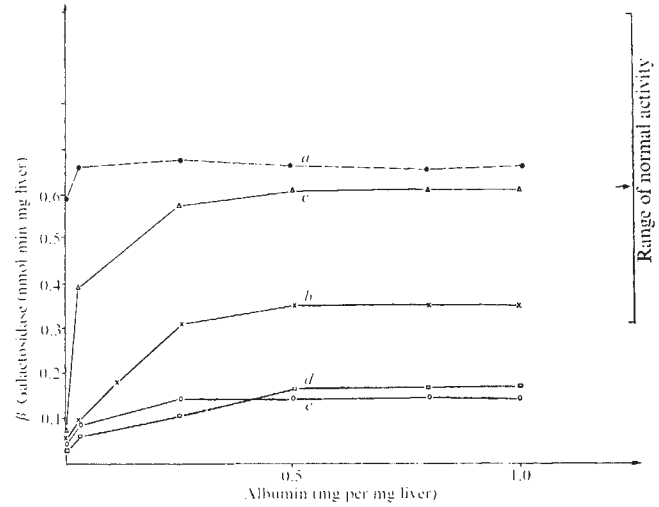


Fig. 2 Influence of increasing amounts of albumin on β-galactosidase activity in liver homogenates from *a*, control; *b*, Hunter; *c–e*, Hurler individuals. Legend and methods as in Fig. 1, but albumin in acetate buffer (0.2 M, *p*H=4.5) was added to the liver homogenate instead of CPC. The range of normal values is shown at the upper right. The arrow indicates the mean value of the enzyme activity in eight control autopsy livers.

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Aspirin, indomethacin, catecholamine and prostaglandin interactions on rat arterioles and rabbit hearts

VANE *et al.* have demonstrated that antipyretic, anti-inflammatory drugs can inhibit prostaglandin synthesis and release[1–3]. We have investigated the effects of aspirin on the responses of rat arterioles to noradrenaline.

Using male, 150 g, Sprague-Dawley rats anaesthetised with ether we cannulated the superior mesenteric artery, dissected out the mesenteric part of its vascular bed and mounted the preparation in an organ bath[4]. We perfused the artery with Krebs bicarbonate buffer at 37° C, bubbled with 5% carbon dioxide in oxygen. After an equilibration period of 30 min we adjusted the flow rate so that the pressure in the arterial cannula had a mean value of about 80 mm Hg. The flow rate was then left constant for the remainder of the experiment. The baseline pressure remained steady (variation less than 10%) for periods as long as 3 h (Fig. 2). The preparation responds to injection of vasoconstrictor drugs by a rise in arterial pressure. The maximal pressor response to constrictor drugs is usually in the region of 80–120 mm Hg above baseline. Noradrenaline solution (0.1 ml) in 0.9% saline was injected into the arterial cannula at regular intervals. Each preparation was tested with a range of noradrenaline concentrations to find one which gave a pressure rise of about 70 mm Hg. This was usually about 1 ng ml^{-1} solution. This concentration was then used for the rest of the experiment. Each injection produced a transient rise in pressure which returned to baseline within about 1 min. Once three pressor responses of constant amplitude (±10%) had been obtained, the formal experiment was started, results being expressed as percentages of the mean initial response.

Aspirin was added to the perfusate in three concentrations 5, 10 and 20 μg ml^{-1} (Fig. 1). The results demonstrate a reversible depression of responsiveness to noradrenaline, the constrictor effect being eliminated by an aspirin concentration of 20 μg ml^{-1}. Baseline perfusion pressure was not altered.

The abolition of responsiveness might be explained by *p*H changes, a direct action of aspirin itself on the interaction between noradrenaline and muscle receptors, on excitation–contraction coupling or on the contractile process,

or an indirect action of aspirin depending on its suppression of prostaglandin synthesis. The 20 μg ml^{-1} aspirin concentration produced a *p*H change of less than 0.1 unit and changes of 0.3 unit produced by adding acetic acid to the buffer caused no significant changes in responsiveness. In five experiments indomethacin, another suppressor of prostaglandin synthesis, in a concentration of 5 μg ml^{-1} also produced a complete abolition of responsiveness. We therefore attempted to reverse the effect of aspirin by adding prostaglandin E_2 to aspirin-blocked preparations (Fig. 2).

A prostaglandin E_2 concentration of 1 pg ml^{-1} produced partial restoration of responsiveness. Above 10^3 pg ml^{-1} the relationship between E_2 concentration and responsiveness to noradrenaline flattened and no further increase could be demonstrated above 10^4 pg ml^{-1}. Furthermore at an E_2 concentration of 10^4 pg ml^{-1} the two noradrenaline doses used produced essentially the same responses: sample testing with higher noradrenaline concentrations demonstrated that this was a maximal response under these conditions. Restoration of the control perfusion fluid for 30 min showed that the initial control responsiveness could be nearly restored. Addition of prostaglandin $F_2\alpha$ to the perfusion fluid in concentrations up to 100 ng ml^{-1} failed to restore responsiveness. Prostaglandin E_1 had a weak action, being about 100 times less effective than prostaglandin E_2.

Possible explanations for the results are that endogenous prostaglandin synthesis may be necessary for the combination of noradrenaline with its receptor site or for the chain of events which occur between catecholamine–receptor combination and contraction. The mechanism is not specific to alpha receptors since we have also shown that the vasoconstrictor effects of vasopressin and angiotensin can also be abolished by either aspirin or indomethacin and restored by prostaglandin E_2. In contrast in five experiments using a rabbit Langendorff perfused heart preparation the chronotropic and inotropic beta actions of adrenaline were not inhibited by concentrations of indomethacin up to 50 μg ml^{-1}. This raises the interesting possibility that one difference between alpha and beta effects of catecholamines may be that the former require prostaglandins and the latter do not.

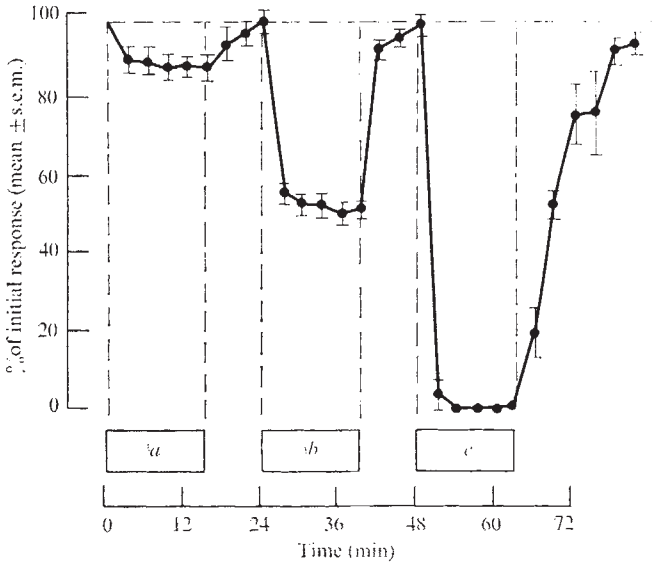


Fig. 1 Changes in responsiveness of the rat mesenteric vascular bed to noradrenaline brought about by adding aspirin to the perfusing fluid. Six experiments were performed. Bars indicate 15 min periods during which aspirin was added to the perfusing fluid; concentrations: *a*, 5 μg ml^{-1}; *b*, 10 μg ml^{-1}; *c*, 20 μg ml^{-1}. Before, between and after the bars the preparation was perfused with Krebs-bicarbonate buffer.

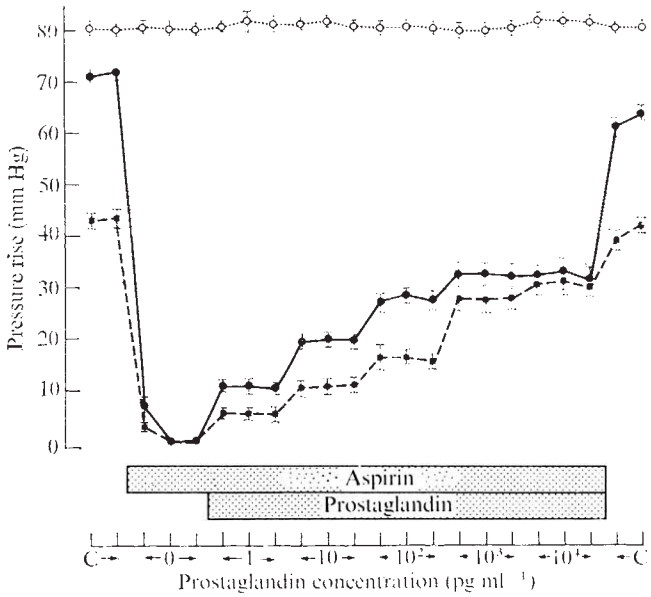


Fig. 2 Responses of the aspirin-blocked rat mesenteric preparation to noradrenaline in the presence of various concentrations of PGE_2. Closed circles indicate responses to 0.1 ml of a noradrenaline concentration sufficient to produce a pressor response in the initial control period of about 70 mm Hg: closed squares indicate responses to 0.1 ml of a noradrenaline solution of half that concentration. During each test the lower concentration was given first and the higher after the first response was completely over. Open circles indicate the baseline pressure. Five experiments were performed and each point represents the mean pressure rise ± s.e.m. The pairs of noradrenaline injections were given at five minute intervals. After initial control (C) responses aspirin was added to the perfusate in a concentration of 30 μg ml^{-1} in order to block responsiveness completely. While maintaining the aspirin perfusion, progressively increasing concentrations of PGE_2 (Upjohn) were added to the perfusate, perfusion of each concentration being maintained for 15 min. At the end of the experiments Krebs-bicarbonate buffer was perfused through the preparation for 30 min. The last two C (control) responses were the pressor effects obtained 20 and 30 min after returning to the Krebs solution.

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Syntheses of amino acids from aliphatic carboxylic acid by glow discharge electrolysis

Glow discharge electrolysis (GDE)[1,2] has been studied mainly on inorganic compounds such as water, ammonia,

and metal ions in aqueous solutions. Little study has been done, however, on the chemistry of GDE using organic compounds[3–7] although experimental results indicate that the application of GDE to organic compounds is a promising area in chemistry and is especially interesting in connection with the concept of chemical evolution.

Here we report representative results of amino acid formation by contact glow discharge electrolysis (CGDE)[1,2,8] from several aliphatic carboxylic acids by direct amination using ammonia in aqueous solution.

Two types of electrolysis tubes were used for CGDE; one a U-shaped tube, the other a single tube. In the U tube, the cathode and anode compartments were separated by a fine-porosity glass frit and CGDE was carried out in the

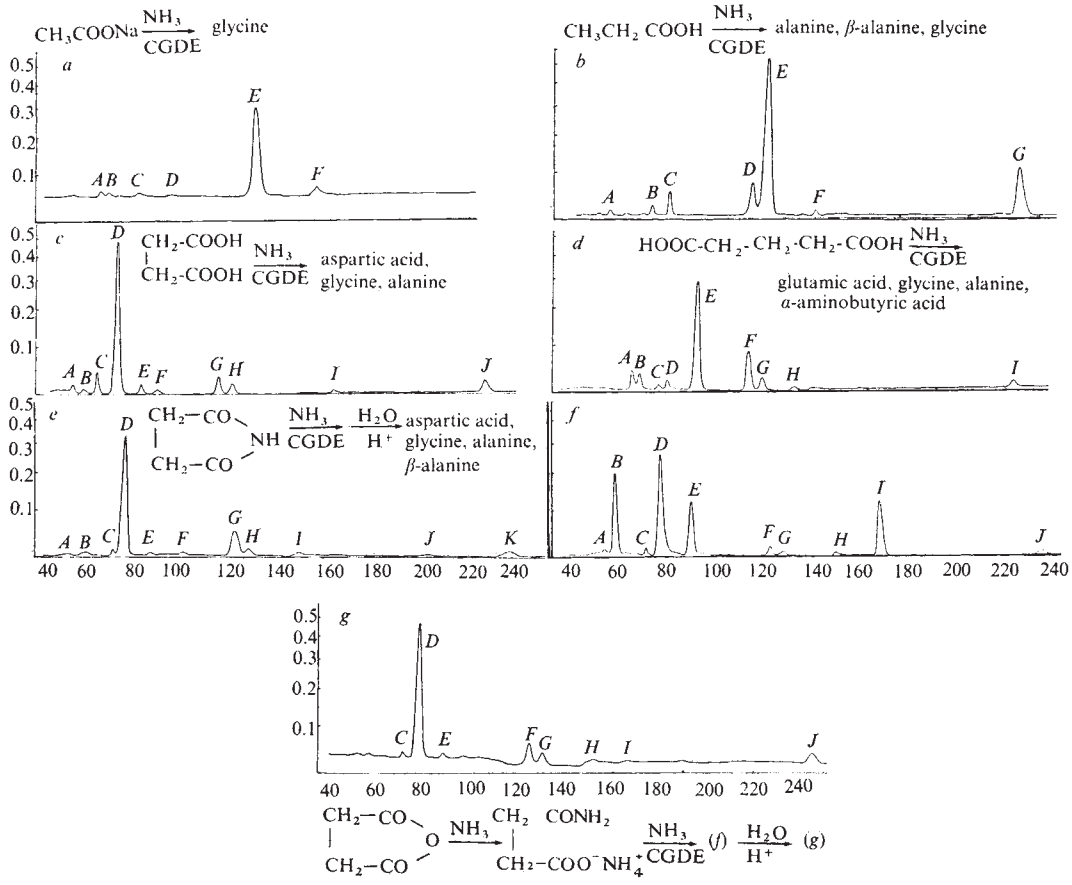


Fig. 1 *a*, *A*, *B*, *C*, *D*, unknown; *E*, glycine; *F*, unknown + buffer change. Reaction conditions: electric current, 65 mA; reaction temperature, 15° C; reaction time, 6 h. The yield of glycine (peak *E*) was 3.5% from sodium acetate. *b*, *A*, *B*, *C*, unknown; *D*, glycine; *E*, alanine; *F*, unknown; *G*, β-alanine. Reaction conditions: electric current, 75 mA; reaction temperature, 15° C; reaction time, 3 h. Alanine (peak E, yield 6.9%) was synthesised by amination of the α-carbon of propionic acid. β-Alanine (peak *G*, yield 5.3%) was formed by amination of the β-carbon. A small amount of glycine (peak *D*) was formed by the splitting of the α–β carbon linkage. The glycine formation by α–β cleavage of carboxylic acid is a general phenomenon in GDE experiments. *c*, *A*, *B*, *C*, unknown; *D*, aspartic acid; *E*, *F*, unknown; *G*, glycine; *H*, alanine; *I*, unknown; *J*, β-alanine. Reaction conditions: electric current, 50–70 mA; reaction temperature, 15° C; reaction time, 6 h. The major amino acid product is aspartic acid (peak *D*, yield 9.1%). Glycine (peak *G*, yield 0.5%) was formed by α–β bond cleavage. Alanine (peak *H*, yield 0.3%) was formed by β-decarboxylation, and β-alanine (peak *J*, yield 1.6%) was formed by α-decarboxylation of aspartic acid. In the several aspartic acid formations, a peak corresponding to β-hydroxy aspartic acid (peak *C*) was usually found with aspartic acid. The nature of the amino acid at peak *C*, however, is not yet fully characterised. Similarly, peaks corresponding to threonine and serine (peaks *E* and *F*) were commonly found in the reaction products of GDE experiments. These are also not yet characterised. *d*, *A*, *B*, *C*, *D*, unknown; *E*, glutamic acid; *F*, glycine, *G*, alanine, *H*, α-aminobutyric acid; *I*, unknown. Reaction conditions: electric current, 75 mA; reaction temperature, 15° C; reaction time, 3 h. The main product was glutamic acid (peak *E*, yield 3.1%). Glycine (peak *F*, yield 0.8%) and alanine (peak *G*, yield 0.2%) were also formed. The peak of an expected product, β-aminoglutaric acid, was not identified. *e*, *A*, *B*, *C*, unknown; *D*, aspartic acid; *E*, *F*, unknown; *G*, glycine; *H*, alanine; *I*, *J*, unknown; *K*, β-alanine. Reaction conditions: electric current, 75 mA; reaction temperature, 5° C; reaction time, 3 h. After the reaction was over, the ammoniacal solution was evaporated under reduced pressure, and the residue was hydrolised with 6 N hydrochloric acid for 6 h. The main product was aspartic acid (peak *D*, yield 3.2%). Glycine (peak *G*, yield 0.8%) and alanine (peak *H*) were also found in the hydrolysate. The peak *K* corresponds to that of β-alanine (yield 0.7%). *f*, *A*, *B*, *C*, unknown; *D*, aspartic acid; *F*, glycine; *G*, alanine; *H*, buffer change; *I*, isoasparagine; *J*, β-alanine. *g*, *C*, unknown; *D*, aspartic acid; *E*, unknown; *F*, glycine, *G*, alanine; *H*, buffer change; *I*, trace of isoasparagine; *J*, β-alanine. Reaction conditions: electric current, 55 mA; reaction temperature, 5° C; reaction time, 3 h. The amino acids in the reaction mixture were analysed without hydrolysis (*f*). Asparagine (peak *E*, yield 1.1%) and isoasparagine (peak *I*) were formed by CGDE. A larger aspartic acid peak however (peak *D*, yield 2.6%), was found. This suggests that the CGDE process could hydrolyse the amide bond of the asparagine and isoasparagine formed. Glycine (peak *F*, yield 0.1%), alanine (peak *G*), and β-alanine (peak *J*) were also observed. The nature of the large peak *B* is not known; however, the peak *B* disappeared after acid hydrolysis (*g*). The yields of aspartic acid, glycine, alanine, and β-alanine after hydrolysis were 4.5, 0.5, 0.3, and 1.2%, respectively.

anodic compartment. In the amination reaction of carboxylic acid (0.0025 mol in 10 ml of concentrated aqueous ammonia), CGDE took place in conditions of ammonia gas saturation and stirring. All reactions were carried out at 1 atm. The reaction temperature was measured by thermometer in the reaction tube which was immersed in the methanol–dry ice bath. The applied electric current (d.c.) was 450–600 V and 20–100 mA. The reaction time ranged from 0.5–6 h. After the reaction ceased, the reaction mixtures were evaporated to dryness under reduced pressure and the residues were appropriately diluted for amino acid analysis. Some of the reaction products were hydrolysed with hydrochloric acid and the amino acids were then analysed. The reaction mixture was also treated with 2,4-dinitrofluorobenzene, and the resulting dinitrophenyl (DNP)-amino acids were separated by column chromatography and thin layer chromatography. The major amino acid products were confirmed as DNP-amino acids.

Figure 1 represents amino acid formation from acetic acid, propionic acid, succinic acid, glutaric acid, succinylanhydride, and succinimide. The resulting amino acids, their yields, and the reaction conditions are described. The yields of amino acids in some amination reactions reached up to 13% (Fig. 1*b*). These amino acid formations by CGDE do not, however, represent optimal conditions.

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Facilitation of lysosome disruption by ATP at low *p*H

HAYASHI *et al.*[1] reported that ATP stimulates *in vitro* the degradation of proteins at *p*H 4.5 by lysosomes which were intact at the start of the experiment. In accordance with our previous results, no effect was found at *p*H 5.0 (ref. 2). Although they found more rapid degradation by disrupted lysosomes, these authors concluded that the effect of ATP was not due to an increased labilisation of the lysosomal membrane, but that "ATP facilitates the pinocytotic engulfment of proteins into lysosomes and thereby stimulates proteolysis". This conclusion was mainly based on experiments on non-sedimentable cathepsin D activity, discussed below. We have reinvestigated the effect of ATP on protein breakdown at different *p*H values, and found essentially the same results. Our evidence shows, however, that the increase of proteolysis is due to facilitation of lysosome disruption by ATP at *p*H 4.5.

Figure 1 shows the degradation of haemoglobin using intact and disrupted mitochondrial-lysosomal fractions prepared according to Sawant *et al.*[3]. Similar results were obtained with a mitochondrial-lysosomal fraction prepared according to Bouma and Gruber[5] and with a fraction isolated from hepatocytes[6]. ATP, in the presence of Mg^{2+} ions, stimulated the degradation of haemoglobin by the intact particle fraction at *p*H 4.48, but not at *p*H 4.73 and *p*H 5.05. During the first 30 min, the stimulation at *p*H 4.5 was two to three times in several experiments. The same results were obtained for the

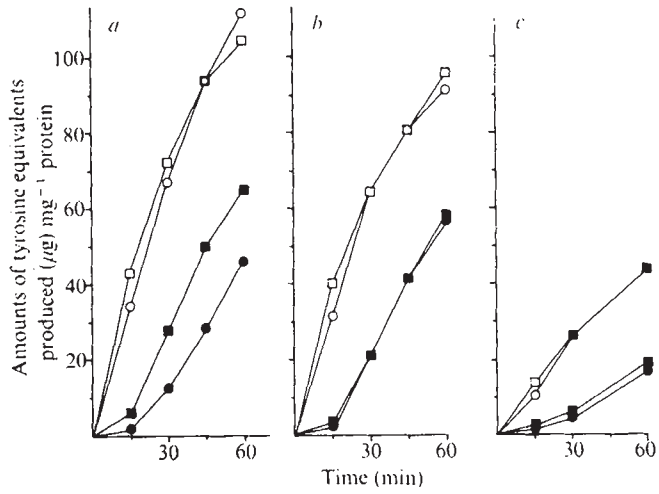


Fig. 1 Haemoglobin degradation by intact and disrupted lysosomes. A mitochondrial-lysosomal fraction was isolated from rat liver according to Sawant *et al.*[3]. The particle fraction was used either intact, or after disruption by three cycles of freezing in liquid nitrogen and thawing at 38° C. The incubation mixture contained 2.5 mg mitochondrial-lysosomal protein, 10.8 mg haemoglobin, 50 mM Na-acetate buffer and 0.25 M sucrose in a volume of 3.1 ml. The incubation temperature was 38° C. ATP and $MgSO_4$, were used at concentrations of 10 mM. At the times indicated, samples of 0.5 ml were added to 1.0 ml ice-cold 10% trichloroacetic acid. The mixture was cooled immediately and centrifuged at 0° C. Degradation products were determined in the supernatant by means of the Folin–Ciocalteu reagent, using tyrosine as standard[4]. ●, Intact lysosomes, no additions; ■, intact lysosomes, ATP and Mg^{2+} present; ○, disrupted lysosomes, no additions; □, disrupted lysosomes, ATP and Mg^{2+} present. Degradation at *a*, *p*H 4.48; *b*, *p*H 4.73; *c*, *p*H 5.05.

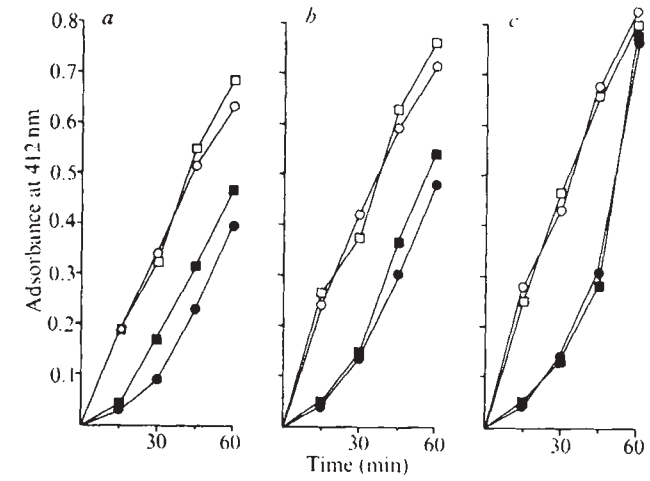


Fig. 2 Hydrolysis of glycyl-phenylalanyl-*p*-nitroanilide by intact and disrupted lysosomes. The incubation conditions are the same as those described under Fig. 1, except for the inclusion of 10 mM NaCl, 9 mM dithiothreitol and 0.315 mM GPNA. The trichloroacetic acid supernatants were added to 0.2 ml 2.0 M Tris-HCl buffer *p*H 9.0, and the adsorbance was determined at 412 nm. ●, Intact lysosomes, no additions; ■, intact lysosomes, ATP and Mg^{2+} present; ○, disrupted lysosomes, no additions; □, disrupted lysosomes, ATP and Mg^{2+} present. Degradation at *a*, *p*H 4.45; *b*, *p*H 4.78; *c*, *p*H 5.00.

hydrolysis of glycyl-phenylalanyl-*p*-nitroanilide (GPNA), a low-molecular weight substrate of cathepsin C (ref. 7), as is shown in Fig. 2. These findings are most simply explained if one assumes that ATP facilitates the disruption of the lysosomal membrane at *p*H 4.5.

Hayashi *et al.*[1] rejected this explanation because ATP did not enhance the non-sedimentable cathepsin D activity. Non-sedimentable activity is, however, not always a valid reflection of lysosomal integrity[8], since at low *p*H values some enzymes are strongly adsorbed to membranes of disrupted lysosomes[9]. For that reason, we investigated the effect of ATP on the 'free' activity of cathepsin C. The results are shown in Table 1. The presence of ATP clearly accelerates lysosomal disruption at *p*H 4.5 only. It should be noted, that at the times indicated in the Table, the cathepsin C assay, which itself lasted 15 min, was started. ATP was present in both incubation systems during the assay. Thus, the relative increase in labilisation at *p*H 4.5, due to the presence of ATP during the incubation, is larger than that indicated by the data of Table 1.

Table 1 Influence of ATP on the 'free' cathepsin C activity of a mitochondrial-lysosomal fraction during incubation at *p*H 4.5, 4.8, and 5.0

Time (min)	'Free' cathepsin C activity (%)					
	*p*H 4.48		*p*H 4.78		*p*H 5.00	
ATP	−	+	−	+	−	+
0	25	29	24	24	26	26
10	58	78	41	43	42	37
20	86	87	65	66	59	54
30	94	96	82	89	82	79
60	97	102	91	89	100	98

A mitochondrial-lysosomal fraction was prepared according to Sawant *et al.*[3] and incubated as described under Fig. 1. At the times indicated, samples of 0.5 ml were added to 0.5 ml buffered isotonic GPNA solution (final concentrations: 50 mM sodium acetate buffer, 10 mM Cl^-, 9 mM dithiothreitol, 0.315 mM GPNA and 0.25 M sucrose). The chloride ions and the thiol were added to activate and stabilise cathepsin C (ref. 10). To those samples which had been preincubated without ATP and MgSO4, these substances were added together with the GPNA solution to give the same final concentration of 5 mM. After 15 min at 38° C, the incubation with GPNA was stopped by adding 0.2 ml trichloroacetic acid to a final concentration of 10%. The trichloroacetic acid supernatant was added to 0.2 ml 2M Tris-HCl buffer *p*H 9.0, and the adsorbance was determined at 412 nm in 2 cm cells. Blanks were prepared by adding trichloroacetic acid before the enzyme. The 'free' cathepsin C activity, determined in this way, is expressed as a percentage of the value obtained after three cycles of freezing–thawing (see text under Fig. 1). Three representative experiments are shown; +, the presence of 10 mM ATP and 10 mM Mg^{2+}; −, their absence during incubation.

At *p*H 4.8 and 5.0 ATP has no effect on lysosomal membrane stability. At *p*H 7.0, ATP even stabilises the lysosomal membrane[11,12]. Thus, the direction and the magnitude of the effect of ATP on the lysosomal membrane is strongly *p*H dependent.

This is reflected in the absence of a stimulating effect of ATP on the degradation of haemoglobin and GPNA at *p*H 4.75 and 5.0. The *p*H dependence explains why Hayashi *et al.*[1] found no effect on acid phosphatase and β-glucuronidase activity measured at *p*H 4.8 and 5.2 respectively.

The second argument of Hayashi *et al.*[1] for the hypothesis that ATP stimulated the uptake of macromolecules into lysosomes, was the finding that ATP increased the amount of non-washable cytosol protein in the mitochondrial-lysosomal pellet slightly. A control experiment with a disrupted fraction was, however, not carried out. We found that even at neutral *p*H adsorption of cytosol proteins was as good to a disrupted as to an intact mitochondrial-lysosomal fraction.

Thus the effects of ATP can be explained by its strongly *p*H-dependent influence on the stability of the lysosomal membrane. No valid evidence for the uptake of macromolecules into lysosomes at *p*H 4.5 exists.

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Inhibition of high-affinity glial uptake of ^{14}C-glutamate by folate

Evidence is accumulating to support the suggestion[1] that folic acid (pteroyl glutamic acid) may play a role in epileptic phenomena. *Grand mal* epileptic patients undergoing anticonvulsant drug therapy are likely to develop megaloblastic anaemia as a result of disturbances in folic acid metabolism[1], and it has been reported that correction of the folic acid deficiency by folate therapy results in an exacerbation of the frequency and intensity of epileptic episodes[2,3]. It is also known that folate and related pteridines possess convulsant properties in their own right when administered in high intravenous doses to rats, or given intracortically[4].

In a recent examination[5] of the effects of iontophoretically applied folate or folinate (N^5-formyl—5, 6, 7, 8—tetrahydrofolate) on the firing rate of neurones in the cat pericruciate cortex, these substances were found to have powerful facilitatory effects on the firing of spontaneously active, or glutamate-activated cells though they possessed only weak excitatory actions on quiescent neurones. The mechanism of the facilitatory action was not clear, but the authors provided some evidence for a possible blockade of inhibitory γ-aminobutyric acid (GABA) receptors. Similarly, it has been found that folate will antagonise presynaptic inhibition in the cuneate nucleus[6]; however, no antagonism of GABA-induced inhibition was demonstrable[7].

Glutamate is likely to be an important excitatory neurotransmitter in the CNS[8], and the major means of terminating the synaptic action of this amino acid is considered to be by re-uptake, largely into glial cells[9]. The rat dorsal root ganglion provides a convenient model system for studying the glial uptake of amino acids and has been previously used[10] to demonstrate an active, energy-dependent, high-affinity transport process ($K_m = 2 \times 10^{-5}$ M) for glutamate.

Here I examine the possibility that folate might interfere with this glutamate uptake mechanism.

Rat dorsal root ganglia were isolated and desheathed, and transferred in pairs to 0.75 ml of Krebs-bicarbonate medium (*p*H 7.4) and preincubated for 10 min at 27°C under O_2-CO_2 (95 : 5). L-U-^{14}C-glutamate (Amersham) was then added to give a final concentration of 10^{-6} M, and the incubation con-

tinued for a further 15 min. At the end of this time ganglia were rapidly rinsed in ice-cold Krebs medium, blotted on filter paper and weighed individually. The tissues were transferred to liquid scintillation vials, solubilised with 400 μl Protosol (NEN Chemicals) and total radioactivity assayed by liquid scintillation counting. Uptake of ^{14}C-glutamate was found to be linear with time. In each series of experiments ganglia were included which were incubated throughout at 0° C. The radioactivity in these blanks, which enabled a correction to be made for the ^{14}C-glutamate localised in the extracellular water, and for uptake due to diffusion, was subtracted from the values in the corresponding experimental sample (in each case the radioactivity in the blanks was less than 16% of the control). ^{14}C-glutamate was accumulated by dorsal root ganglia at a rate of 5.86 ± 0.33 pmol per mg tissue per 15 min (means ± s.e.m. of 10 independent observations).

The effects of folate (Sigma, UK), the related pteridine, folinate (calcium salt; Lederle) and the dihydrofolate reductase inhibitor, methotrexate, 4-amino-N^{10}-methylpteroyl glutamic acid (Lederle) were examined at a single test concentration of 5×10^{-4}M, on the uptake of 10^{-6}M ^{14}C-glutamate. Each of the three compounds tested produced an inhibition of 38.8 ± 4.9%, 35.5 ± 6.4% and 27.2 ± 1.9% respectively (means ± s.e.m. of eight independent observations).

The Michaelis-Menten kinetics were determined for folate, using a limited range of folate concentrations (2.5×10^{-4}M–10^{-3}M). The values are presented as a Dixon plot (Fig. 1) which indicates that folate inhibits competitively the high-affinity uptake of ^{14}C-glutamate with a $K_i = 5.64 \times 10^{-4}$M.

The effects on the uptake process of a clinically useful anticonvulsant, diphenylhydantoin, were examined at a concentration of 5×10^{-4}M. The compound itself was without effect on the uptake of ^{14}C-glutamate, nor did it affect the inhibition of uptake by folate.

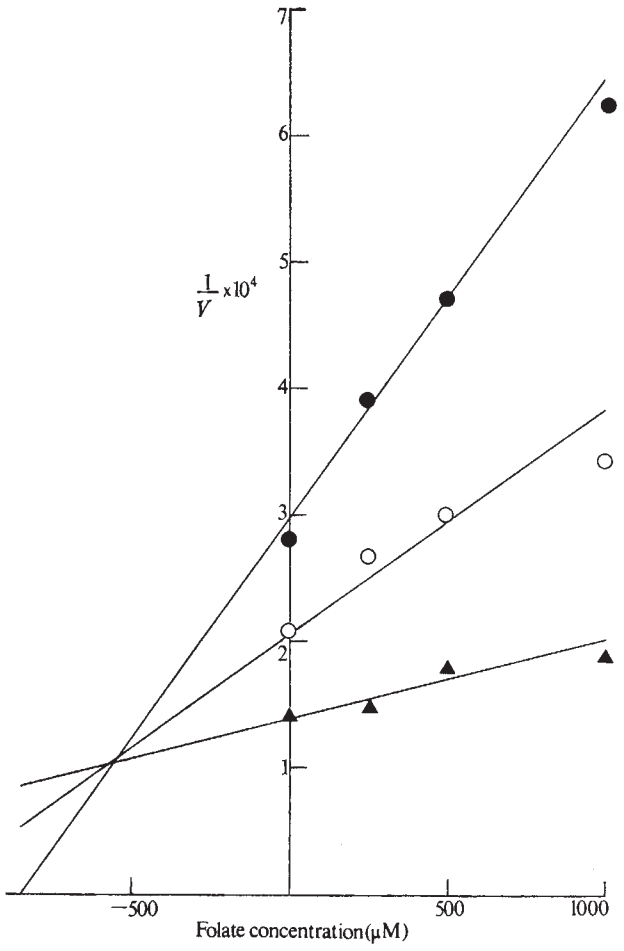


Fig. 1 Dixon plot for the effect of folate on uptake of ^{14}C-glutamate by dorsal root ganglia. V = d.p.m. per mg per 15 min. ^{14}C-glutamate concentrations: ●, 1 μM; ○, 1.5 μM; ▲, 2 μM.

The significance of the inhibition of glutamate uptake by folate seen in these experiments, in relation to the epileptic seizure, and to the effects of the therapeutic administration of folate, is not clear. It seems unlikely that extracellular folate concentrations obtained following even prolonged therapy would be sufficient to markedly influence the re-uptake of glutamate from the synapse. But folate is largely localised in synaptosomal fractions[11], and it has been suggested[12] that focal epileptic discharges may result from an accumulation of folate in the extracellular space around the focus.

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Presynaptic inhibition at mammalian peripheral synapse?

STIMULATION of sympathetic nerves leading to the intestine causes a reduction in intestinal motility[1]. Norberg[2] showed that these nerves ramify mainly in the enteric plexuses and has suggested that inhibition may be exerted at a neural rather than at a muscular level but this suggestion has been disputed[3]. Neural inhibition has been shown to occur by the postsynaptic action of transmitter[4] but at some synapses it has been established that transmission may be inhibited presynaptically[5].

Here we describe experiments directly supporting Norberg's suggestion and which suggest that sympathetic inhibition of synaptic transmission in the myenteric plexus of the guinea pig may be mediated presynaptically rather than by the postsynaptic action of inhibitory transmitter.

Intracellular recordings were made from myenteric neurones in flaps of plexus attached to the anal end of an adjacent segment of guinea pig mid small intestine[6]. The periarterial nerves leading to the intestinal segment were drawn into a suction electrode and stimulated electrically. Synaptic activity was evoked in the myenteric plexus by use of a pair of fine platinum electrodes, one under and the other just above the plexus flap[7]. In some experiments synaptic activity was evoked by distending a balloon placed inside the lumen of the gut segment[6]. Preparations were bathed in modified Krebs solution maintained at 35° C; atropine (2×10^{-7} g ml^{-1}) was present during each experiment to prevent cholinergic excitation of the smooth muscle[6].

In most experiments, trains of 10–100 stimuli (pulse width 0.1 ms, frequency 0.1–50 Hz) applied to periarterial nerves

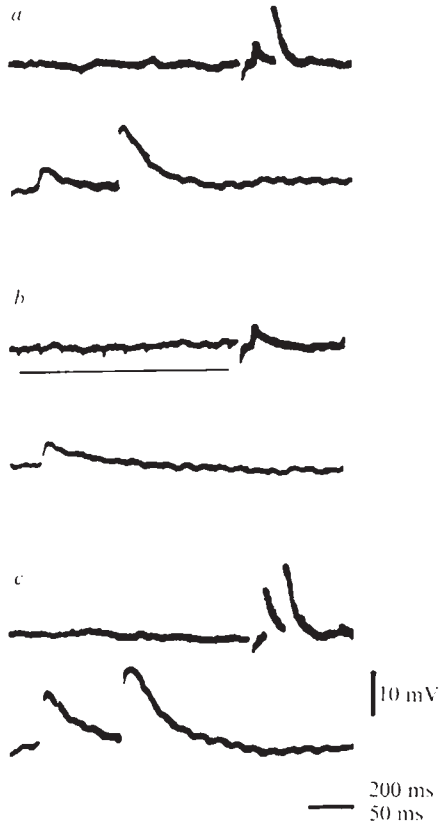


Fig. 1 Effect of periarterial nerve stimulation on transmission in the myenteric plexus of guinea pig small intestine. The upper trace in each illustration records the membrane potential of a myenteric neurone at slow scan speed (horizontal calibration 200 ms); the lower traces show EPSPs evoked by single transmural stimuli applied across the plexus flap close to the recording site (horizontal calibration 50 ms). In *a* and *c*, recorded respectively 15 s before and 15 s after *b*, it can be seen that such stimuli each evoked two EPSPs. But when the periarterial nerves leading to the plexus flap were stimulated (solid bar, 10 Hz for 1 s) just before a transmural stimulus (*b*) only a single EPSP was evoked. Vertical calibration bar (10 mV) applies to each trace.

produced no detectable change in the electrical properties of the myenteric neurones. Transmural stimulation of the flap of myenteric plexus never evoked inhibitory postsynaptic potentials; moreover Nishi and North[8] have shown that noradrenaline, the presumptive transmitter released by periarterial nerves to the intestine, has no effect on the sensitivity of myenteric neurones to acetylcholine (ACh). In two out of more than 40 experiments, short trains of impulses (10 Hz for 1 s) applied to periarterial nerves caused a persistent discharge of excitatory postsynaptic potentials (EPSPs). Such EPSPs were identical to those that could be evoked by close transmural stimulation of a plexus flap and are presumably mediated by the release of ACh[7,8]. We take this observation to support the view that periarterial nerves to the small intestine contain a small proportion of vagal parasympathetic fibres[9].

An intermittent discharge of EPSPs was recorded from some neurones in the absence of any apparent stimulus[6]. Trains of stimuli (2 s duration, 5–10 Hz) applied to the periarterial nerves frequently caused either a reduction in the frequency of occurrence of EPSPs or totally abolished the discharge of EPSPs for several seconds (2–7 s). Distension of the intestinal segment by inflation of the intraluminal balloon increased the frequency of discharge of EPSPs or evoked a discharge of EPSPs in quiescent neurones. Such a discharge of EPSPs was reduced or abolished by a concomitant train of stimuli to the sympathetic nerve. These myenteric neurones mediate either inhibition or excitation of adjacent smooth muscle layers[10]; evidently sympathetic nerve stimulation may depress transmission in both inhibitory and excitatory pathways.

Close transmural stimulation evoked a single EPSP or a cluster of EPSPs; a preceding train of stimuli applied to periarterial nerves either abolished the synaptic response or reduced the number EPSPs in the cluster. If the EPSPs were sufficiently synchronous to evoke an action potential, preceding sympathetic nerve stimulation reduced the synaptic response so that only subthreshold EPSPs were recorded. In some experiments, it was possible to evoke two EPSPs and to selectively abolish one of these by sympathetic nerve stimulation. Such an experiment is illustrated in Fig. 1. It can be seen that the second EPSP was abolished in an 'all-or-none' manner whilst the first EPSP was unaffected. In other experiments early EPSPs were abolished rather than late EPSPs. The scatter of latencies for EPSPs could result from delays due to transmission through interneurones but this seems unlikely as EPSPs were repeatedly evoked during repetitive transmural stimulation (10 Hz); polysynaptic nerve pathways in the myenteric plexus often failed to transmit at frequencies as low as 0.2 Mz (ref. 6).

When intracellular recordings were made from longitudinal or circular muscle, trains of stimuli (20 Hz for 5 s) applied to periarterial nerves produced no detectable change in membrane potential of cells of longitudinal layer. They did, however, produce a detectable hyperpolarisation (5–10 mV) of circular muscle membrane potential; frequencies less than 20 Hz only occasionally produced a small hyperpolarisation (1–3 mV) of this layer.

The simplest explanation for our findings is that sympathetic inhibition is mediated presynaptically. In most experiments repetitive sympathetic nerve stimulation reduced the number of EPSPs in a cluster of synaptic potentials. As the amplitude of the EPSPs which persisted during periarterial nerve stimulation was not reduced, it seems unlikely that inhibition is mediated by a conductance change in the postsynaptic membrane. The possibility that inhibition is mediated postsynaptically at restricted areas some distance from the cell soma, however, cannot be completely excluded. It would also seem from these experiments that the mechanism of sympathetic inhibition is somewhat different to that observed at the crayfish neuromuscular junction, as EPSPs were abolished in an 'all-or-none' manner rather than by a reduction in quantal content[5]. Nevertheless, these experiments show that sympathetic nerve stimulation may inhibit transmission in the myenteric plexus and may block local reflexes activated by distension of an intestinal segment.

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Conductance increase by adrenaline in guinea pig taenia coli studied with voltage clamp method

USING the double sucrose gap method, Bülbring and Tomita[2] have shown that adrenaline hyperpolarises the membrane and reduces the electrotonic potential produced by constant current pulses in the guinea pig taenia coli muscle. They concluded, from the effects of membrane polarisation and changing external ionic composition on the adrenaline action, that an increase in the K and Cl conductances of the membrane is responsible for the hyperpolarisation.

This agrees with the results obtained from ionic flux experiments[5]. In contrast, however, it has been recently reported, on the basis of voltage clamp experiments, that adrenaline increases the negativity of the potassium equilibrium potential without causing significant changes in the membrane conductance[4]. As the voltage–current relation is roughly linear[1,3,4], it is difficult to reconcile these different conclusions.

We therefore examined the effect of adrenaline using the current clamp and voltage clamp methods in the same preparation. The double sucrose gap apparatus was essentially the same as that previously used[2], although of slightly smaller dimensions, that is, the centre pool where the test solution flowed, was 700 μm in width. The vertical amplifier of the oscilloscope (Textronix, 3A7) was used as a feedback amplifier for the voltage clamp experiments and electrical responses were recorded with a rectilinear pen recorder (Nihon Kohden, PMP–3004).

Figure 1 shows effects of adrenaline (10^{-6} g ml^{-1}) injected into the stream of Krebs solution flowing through the centre pool, in current clamp (Fig. 1*a*) and voltage clamp (Fig. 1*b*) conditions. In Fig. 1*a*, a hyperpolarising current pulse (2 s duration) of constant intensity (5×10^{-7} A) was applied every 8 s. The previous observation[2], that the membrane was hyperpolarised and the electrotonic potential was concomitantly reduced by adrenaline, was confirmed. When adrenaline was applied in the voltage clamp condition, an outward current was observed and in response to hyperpolarising voltage pulses the membrane currents were increased (Fig. 1 *b*). Thus, it is clear that adrenaline increases the membrane conductance and produces an outward current.

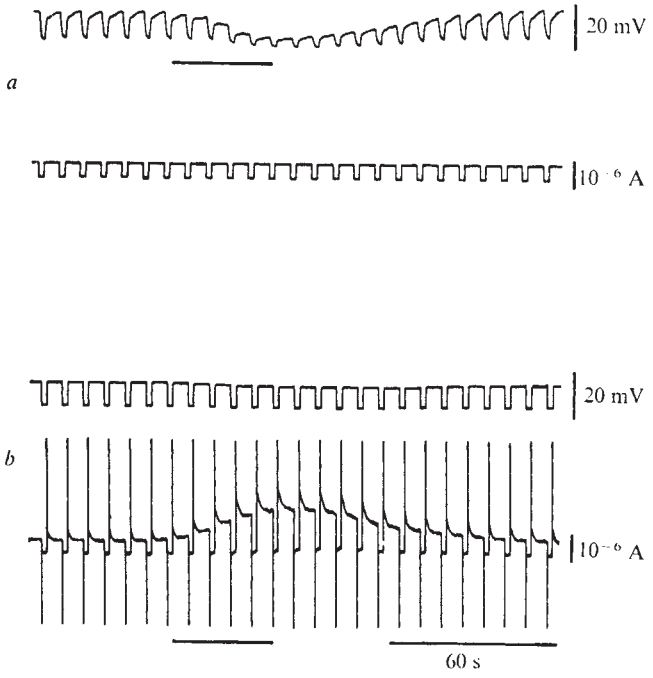


Fig. 1 Effects of a short application of adrenaline (10^{-6} g ml^{-1}) on the guinea pig taenia coli. Adrenaline infusion is indicated by the horizontal bar. Top trace, membrane potential, downward deflection indicating hyperpolarisation; bottom trace, membrane current, downward deflection indicating inward current. *a*, Current clamp; *b*, voltage clamp.

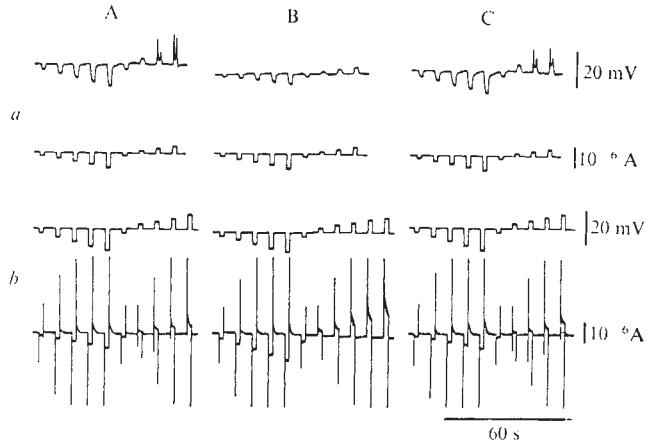


Fig. 2 Effects of a prolonged application of adrenaline (B) and its recovery (C). Compare with control experiments (A). *a*, Current clamp; *b*, voltage clamp. Note decrease in electrotonic potential (top trace) in *a*, and increase in membrane current flowing during the voltage pulses (bottom trace) in *b* during adrenaline application. Also note that an early transient inward current produced by depolarising voltage pulse was abolished in B.

This was also confirmed by studying the current–voltage relationship (Fig. 2 *a*) and the voltage–current relationship (Fig. 2 *b*) in the absence and presence of adrenaline (5×10^{-7} g ml^{-1}). After taking control records in current clamp and voltage clamp conditions in normal Krebs solution, adrenaline was continuously administered and 10 min later the same experiments were repeated in the presence of adrenaline. Records were taken 10 min after washing out the adrenaline. There was some gradual recovery in membrane potential and membrane resistance during prolonged exposure to adrenaline. Even after more than 10 min exposure to adrenaline, however, it was clear that the membrane was still hyperpolarised, the membrane resistance reduced, and the spike or the early transient inward current blocked. Thus, the results were similar to those obtained by a short application of adrenaline.

It was also demonstrated that the outward current produced by adrenaline was increased during conditioning depolarisation and reduced during conditioning hyperpolarisation. When the membrane potential was clamped at a hyperpolarised level of more than 15 mV beyond the resting potential, adrenaline produced an inward current.

Our results support the idea that adrenaline increases the membrane conductance, and hyperpolarises the membrane by increasing the outward current. As no careful examination was made of tail currents following repolarisation from a large depolarisation to different voltage levels, a shift in the potassium equilibrium potential is not ruled out by the present experiments. As far as the conductance change is concerned, however, our results conflict with the data obtained by Inomata and Kao[4]. The reason for their failure to demonstrate the conductance change by adrenaline is not clear.

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Development of polycystic ovaries in rats actively immunised against T-3-BSA

THE aetiology of the polycystic ovary syndrome of Stein-Leventhal[1] is still unknown. Histologically, the ovaries from Stein-Leventhal patients are comparable in many respects to polycystic rat ovaries which have been observed in sexually mature rats following the administration of testosterone propionate during the neonatal period or continuous exposure of adult rats to a constant light environment[2]. Furthermore, increased *in vitro* conversion of progesterone to androgen and oestrogen has been described in incubations of both human and rat polycystic ovaries[3]. A clear insight into altered ovarian physiology which may precede the development of polycystic ovaries in the rat might therefore provide a better understanding of the human syndrome.

Mahesh and Greenblatt[4] have suggested that the Stein-Leventhal syndrome is caused by altered steroid synthesis in the ovary resulting in an imbalance of the pituitary–ovarian axis and subsequent persistent stimulation of the ovary by follicle stimulating hormone (FSH). A previous report from this laboratory has clearly shown that active immunisation of intact male rats against testosterone-3-(O-carboxymethyl) imino-bovine serum albumin (T-3-BSA) leads to a grossly elevated total serum testosterone concentration with a concomitant decrease in the circulating biologically active free testosterone fraction due to high-affinity binding by anti-testosterone antibodies[5,6]. This situation was reflected in significantly increased serum concentrations of FSH and luteinising hormone (LH). These observations led us to postulate that a similar immunisation of female rats might induce gradual alterations in the endocrine status of the host conducive to the development of polycystic ovaries, thus providing a well controlled model in which aspects of the aetiology of the syndrome can be examined.

Adult female Sprague Dawley rats (18 weeks) used in this study were housed in an air-conditioned room illuminated between 0700 h and 1900 h. Only rats which had shown at least two consecutive 4-d oestrous cycles (determined by daily vaginal smears) were used. Seven experimental animals were immunised against a T-3-BSA conjugate prepared by the method of Erlanger *et al.*[7]. The immunogen (100 μg) was administered as described previously[5] at monthly intervals. Controls were immunised against unconjugated BSA. Fifteen weeks after beginning immunisation, vaginal smears were taken on 8 consecutive days. At this time all animals immunised against T-3-BSA exhibited persistent vaginal cornification whereas controls continued to cycle normally. After 17 weeks, all animals were exsanguinated, serum collected[5] and the ovaries removed and fixed in Bouin's solution. Significant serum antitestosterone antibody titre (greater than 1:20 final dilution)[5] was detected in three of the T-3-BSA immunised animals, ranging between 1:200–1:700. Ovaries removed from these animals bore massive fluid-filled cysts up to 1.5 cm diameter. Following fixation and dehydration the ovaries removed at autopsy were embedded in paraffin wax and serial 5 μm sections cut and stained with haematoxylin and eosin. Microscopic examination of sections from control ovaries (Fig. 1*a*) revealed apparently normal follicular development with abundant corpora lutea. Those from animals which produced anti-T-3-BSA antibodies, however, displayed large distended follicular cysts characterised by almost total absence of a granulosa cell layer; corpora lutea were rarely observed (Fig. 1*b*).

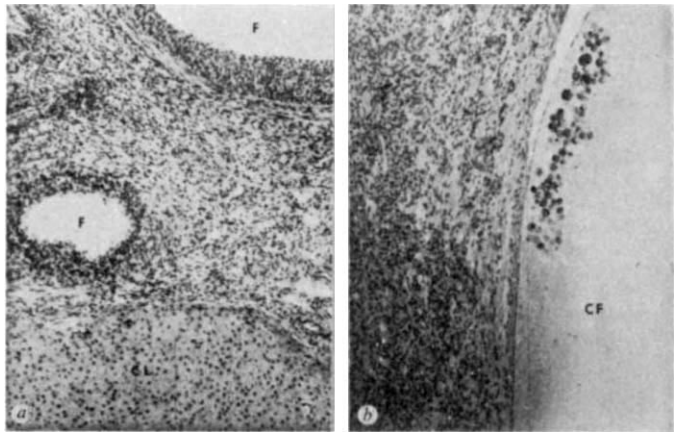


Fig. 1 Photomicrographs of follicles present in, *a*, ovary removed from a control rat immunised against BSA; *b*, ovary removed from a rat immunised against T-3-BSA. F, Normal follicle; CF, cystic follicle; CL, corpus luteum.

The total serum testosterone concentration in these animals, as determined by radioimmunoassay[8] was significantly higher than in controls (Table 1), but using an equilibrium dialysis technique described previously[5], the circulating biologically active[9] free testosterone fraction was found to be significantly reduced (Table 1). Serum concentrations of immunoreactive LH, FSH and prolactin were each determined by radioimmunoassay as before[5] and the values obtained are shown in Table 1. While experimental FSH values were significantly elevated neither LH nor prolactin differed from controls.

These data indicate that a reduction in the circulating free

Table 1 Serum concentration of testosterone, LH, FSH and prolactin in female rats with polycystic ovaries 17 weeks after commencing immunisation against T-3-BSA

	Testosterone (ng per 100 ml) Total	Testosterone (ng per 100 ml) Free	LH (ng ml^{-1})	FSH (ng ml^{-1})	Prolactin (ng ml^{-1})
Controls (immunised against BSA; $n = 6$)	27 ± 18*	9.8 ± 2.5	75 ± 17	465 ± 186	123 ± 114
Experimental (immunised against T-3-BSA; $n = 3$)	90 ± 23	1.72 ± 0.68	62 ± 47	1110 ± 127	69 ± 32
P	< 0.0025	< 0.0025	NS	< 0.0025	NS

* Mean ± 1 s.d.

testosterone fraction which occurs in animals immunised against T-3-BSA is reflected in a selectively increased pituitary FSH output which might be a factor in the aetiology of pathological changes observed in the ovaries of animals thus treated. A detailed examination of various parameters of ovarian physiology before the appearance of polycystic ovaries in such animals may be of value in our understanding of the human syndrome.

We thank the Tenovus Organisation for continuing generous financial support and to the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institute of Health, Bethesda, Maryland for the kits which enabled us to measure the rat LH, FSH and prolactin. We also thank Professor K. Griffiths for criticism and advice. This report forms part of a thesis presented by S. G. H. for the degree of PhD in the University of Wales.

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Influence of NAD+ on development of mouse blastocysts *in vitro*

The metabolites pyruvate and lactate are of central importance for the development of preimplanted mouse embryos[1]. One-cell ova cannot develop when lactate is the only energy source[2], but the necessary pyruvate can apparently be provided for the first cleavage division when the incubation medium contains NAD^+ as well as lactate[3]. Cross and Brinster have reported that the development of the mouse embryo was optimal after the first cleavage division when both lactate and pyruvate were present in the medium[4]. Recent biochemical investigations have demonstrated that NAD^+ metabolism can interfere with DNA synthesis: this effect is independent of the function of NAD^+ as a coenzyme of dehyrogenases[5,6]. We have investigated whether NAD^+ supports the development of mouse blastocysts from the two-cell stage embryos by converting lactate to pyruvate, or whether it inhibits this development by interfering with DNA synthesis.

Sexually mature virgin female white inbred mice (8–10 weeks old) were mated for 2–3 h with sexually mature males of the same strain (two females per cage). Females with vaginal plugs were segregated, and the embryos were obtained from these animals 28–30 h later by flushing Culture Medium BMOC–2 (ref. 7) through the oviduct. The embryos were kept during the collecting period in Culture Medium BMOC–2 with lactate and pyruvate at 30–35° C; afterwards they were washed twice with 0.9% NaCl and 0.1% Ficoll (Pharmacia, Sweden) and transferred to 100 μl Culture Medium BMOC–2 which contained lactate or lactate and pyruvate. The medium also contained NAD^+ in various concentrations and was overlayered with silicone oil Tegiloxan 50 (Goldschmidt, Essen) for the cultivation period. The embryos were cultivated essentially as described by Brinster[7].

NAD an NADH were measured with alcohol dehydrogenase and lactate dehydrogenase[8] by fluorescence spectrometry (Zeiss ZFM 4C).

Table 1 Effects of NAD^+ on development and NADH content of two-cell embryos

NAD^+ (mM)	Development to blastocyst stage (%) BMOC-2(+pyruvate)	BMOC-2(−pyruvate)	NADH (mM)*
0	92 (178†)	40 (35)	0
0.23	65 (26)	81 (21)	0.005
1.0	52 (71)	24 (29)	0.04
5.0	28 (50)	10 (20)	0.12

* Concentration after 66 h incubation with BMOC-2 (without pyruvate) with various NAD^+ concentrations.
† Numbers of embryos.

As seen from Table 1 optimal development of the mouse embryos was obtained using the complete Culture Medium BMOC–2 which contained 0.25 mM pyruvate. After 66 h incubation, 92% of the embryos isolated at the two-cell stage had developed to blastocysts but when pyruvate was omitted this rate was only 40%. In medium containing pyruvate, even small NAD^+ concentrations inhibited embryonic development (Table 1). With increasing NAD^+ concentrations this inhibition became stronger and no blastocysts were observed when the culture medium contained 12.5 mM NAD^+.

But, for embryos cultured without pyruvate, development was stimulated by low NAD^+ concentrations (Table 1). With higher NAD^+ levels (1 mM and higher) the formation of blastocysts from the two-cell stage embryos was inhibited as in the pyruvate-containing medium. No development to blastocysts was observed with a NAD^+ concentration of 12.5 mM in the culture medium.

Table 1 also shows that the NADH content of the culture medium at the end of an incubation without pyruvate is dependent on the NAD^+ concentration. The measured NADH concentration increased almost proportionally with the NAD^+ concentration in the investigated range although we can say nothing about the turnover of the NADH. These data clearly demonstrate that lactate can be metabolised to pyruvate under these conditions. We do not know, however, where these reactions could occur: by intracellular dehydrogenases, by dehydrogenases attached to the zona pellucida, or by dehydrogenases released from the embryos into the medium. This is especially problematical since the pyridine nucleotides cannot usually penetrate mammalian cell membranes[9].

Under our experimental conditions the NAD^+ as a coenzyme of dehydrogenases can apparently provide the pyruvate which the embryos require for their development. But, with higher NAD^+ concentrations the second metabolic effect of NAD^+ overcomes this stimulation and its inhibitory effect on proliferation becomes predominant.

This inhibitory effect may be connected with the formation of adenosine diphosophoribose (ADPR)[4] and its polymer and with the binding of ADPR to the chromatin[5,6]. An NAD glycohydrolase (EC 3.2.2.5) activity is found in the preimplanted mouse embryo[10] which could be of significance in this connection. It is also possible that metabolic breakdown products of NAD^+ are responsible for the inhibitory effect. Adenosine is found to act in this way on the development of the mouse embryo (unpublished results). Nolde and Hilz[11] reported that adenosine caused a reduction

in the proliferation of HeLa cells. We are investigating these problems and that of the uptake and metabolism of NAD^+ by the embryo.

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Evidence for sexual fusion and recombination in the dinoflagellate *Crypthecodinium (Gyrodinium) cohnii*

SEVERAL cytological and biochemical features of dinoflagellate chromosomes, such as low histone content[1–3], threadlike appearance under the electron microscope[1,4–6], absence of centromeres[6], the condensed state persisting throughout interphase[1,4–6], unusual DNA[7] and the controversial possibility of multistrandedness[8,9], suggest that the genetics of these organisms might be unusual. We report here initial experiments on the genetics of the heterotrophic marine, coccoid dinoflagellate *Crypthecodinium cohnii*[10] previously believed to be asexual[10]. We have evidence of sexuality, complementation, recombination and a complex life cycle.

Axenic cultures of Puerto Rican and Woods Hole strains of *C. cohnii* (gifts from Drs K. Gold and L. Provasoli, respectively) and a strain which we isolated from Mattapoisett Bay, Massachusetts, were cloned by isolating single cells on agar by micromanipulation. Several clones of each strain were maintained on Gold's medium (GM)[11], either liquid or solidified with agar (1.0–1.5%).

We found that growing cultures have, in addition to reproductive cysts and coccoid individuals of many sizes and stages of development, motile flagellated cells—swarmers—of various sizes[9] (Fig. 1*a*). DNA content per nucleus, also varies widely (Fig. 1*b*) as previously reported briefly, but within one cyst, the two, four or eight nuclei appear to contain equal amounts. Cysts with eight nuclei are rare and have minimal amounts of DNA per nucleus. Cysts with four nuclei also usually contain the minimal amounts while nuclei of binucleate cysts contain either minimal DNA or twice this amount. These results agree with present and previous observations[10,11] of cysts producing one, two, four or eight cells. An average of such widely divergent amounts of DNA, as determined chemically[3,8] is obviously too large if used to indicate a basal DNA content per genome. As we show later, cells of different size and DNA content result, at least in some cases, from cell fusion.

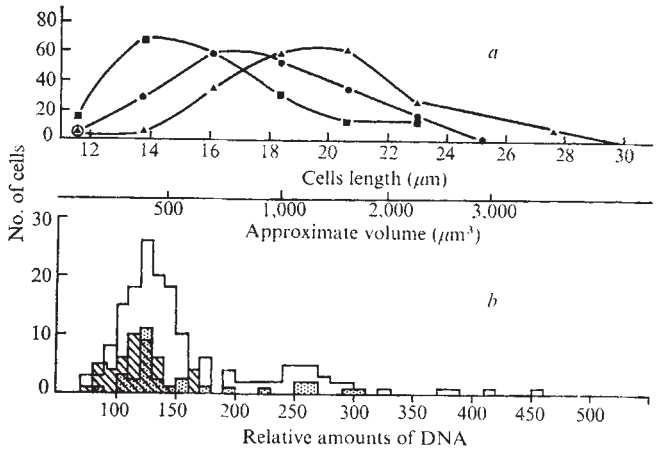


Fig. 1 *a*, Distributions in size of swimming cells of three representative cultures of *C. cohnii*, fm 1 mutant: ■, cells from bottom of log phase liquid culture; ●, cells from top of same culture; ▲, cells from bottom of stationary phase culture. *b*, Measurements of relative amounts of Feulgen dye bound to individual nuclei. A Leitz microspectrophotometer and the two wave length method[14,15] for eliminating distributional error were used. □, Cells of wild type in log phase; ■, in stationary phase; ■, nuclei of different reproductive cysts containing two, four, and eight nuclei.

We used easily distinguishable motility mutants for microscopy and for genetic experiments; one mutant occurred spontaneously and the other was isolated after ultraviolet irradiation. Following *Chlamydomonas* nomenclature[16,17], they were designated fm 1 and fm 2. Motile cells of fm 1 swim at a rate of 50 μm s^{-1}, about one-fifth the normal speed. The swarmers of fm 2 have flagella that appear normal under the light microscope, but locomotion is negligible, with most cells twitching only occasionally.

Extended microscopy of individual normal cells of *C. cohnii* is hampered by their speed. Examination of cultures of fm 1, however, especially cells from the bottom of log phase cultures, revealed motile cells adhering in pairs, moving characteristically with two nonsynchronously beating trailing flagella. (C. K. Franker has observed pairing in other cultures of *C. cohnii*.) With this slow moving strain, it was possible to photograph the pairing of cells and their gradual fusion into a single cell indistinguishable, except for two trailing flagella, from a normal individual of about double the size. (Similar behaviour has been described in other dinoflagellates[18].) Figure 2 shows typical sequences for cells (gametes) of different sizes. Usually fusions involved cells of the same size, but occasionally cells of different sizes fused (Fig. 2*m*, *n* and *s*), and very rarely more than two cells fused. Smaller individuals usually paired side by side, commonly followed by a cruciform juxtaposition of the cells (Fig. 2*a* and *b*). Larger cells frequently completed the process end to end (Fig. 2*j*, *k* and *i*). Fusing cells, watched on open wet mounts until they dried on the glass, and then stained, showed that nuclear fusion closely follows that of the cytoplasm (Fig. 2*q*, *r* and *s*).

Examination of normal cells of *C. cohnii* also revealed pairing and fusion in all clones observed, including those of the Puerto Rican, Mattapoisett and McLaughlin strains. Since all clones represent single cell isolates, it seems likely that the sexuality of *C. cohnii* does not depend on different mating types (homothallism).

In genetic experiments cells of fm 1 were mixed with cells of fm 2 in liquid cultures. After about 10 h normally swimming cells were observed. This was remarkable since mutant cells have never reverted in culture for more than 400 generations, and, since they grow less vigorously than wild type, they would probably soon be overgrown by a back mutation. Fusion of an fm 1 cell and an fm 2 cell could be observed since the trailing flagellum of each could be recognised: that of fm 1 appeared to push both cells while that of fm 2 was ineffectual. Control

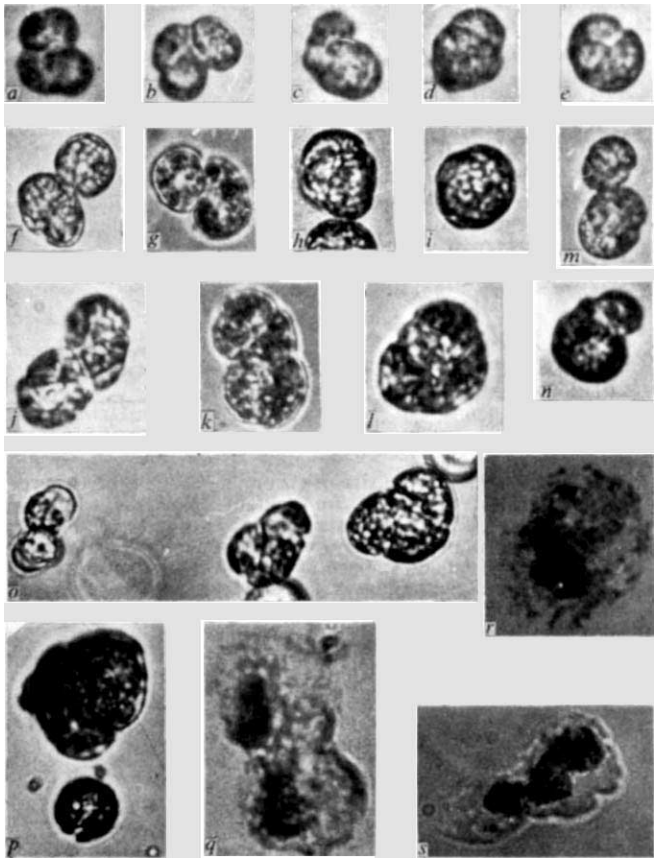


Fig. 2 Pairs of flagellated cells of *C. cohnii*, mutant fm 1. Magnification for living cells, ×528; Feulgen-stained, ×1190. *a–e*, Small cells (length about 14 μm) total time about 35 min; *f–i* larger cells (about 18 μm), total time about 90 min; *j–l*, large cells (about 23 μm), total time more than 12 h; *m* and *n*, anisogamous fusion; *o*, fusions of different sizes occurring at the same time; *p*, fusion product of largest size (about 32 μm); *q*, Feulgen-fast green-stained pair corresponding to (*a*) or (*f*); *r*, similarly stained fusion product corresponding to (*e*) or (*i*); *s*, anisogamous fusion corresponding to (*r*).

experiments in which cells of each mutant were incubated in culture filtrates of the other indicated that no diffusable substance was involved.

In mixtures of fm 1 and fm 2 some normally motile large cells appeared after 6 h, apparently too soon for completion of fusion, encystment, cell division and release of progeny, and we believe that they represent complementation in the 'zygote' itself. Since many fusions are complete within 1 h, the 6 h delay of restored function suggests that complementarily deficient organelles are not contributed by the 'gametes', but that new organelles are elaborated by the 'zygote'. Since cysts can give rise to single motile products, the 'zygote' might encyst for a time during which complementation is effected, after which it emerges as a normally swimming cell. Similar encystment of zygotes has been reported for other dinoflagellates[18].

Only after a minimum of 14 h were the smallest rapidly swimming cells seen in the mixtures. These were too small to be the immediate products of fusion and are thought to be the products of reproductive cysts in which recombinational events occurred. This view is supported by the following genetic evidence. Early appearing large, fast swimming cells ('zygotes') were isolated by micromanipulation on agar. These soon rounded up, grew and ultimately produced four or eight progeny. These were in turn isolated and transferred to liquid medium. After several generations the cells were classified as to their type of motility and their ability to generate normal swimmers in mixtures with their parents (back crosses). Several types of segregations ('tetrads') were observed: in fourteen zygotes analysed four contained only parental mutant types, four showed recombination—each contained two normal swimmers and two double mutants—and the rest appeared to yield parental types phenotypically, but each segregant showed recombination either with both or with neither parent. All segregants have been maintained for many generations in culture without alteration of phenotype, indicating the absence of 'post-meiotic' segregation.

The fact that the mutant characteristics are recoverable from complementing fusion products is consistent with Mendelism, as is the occurrence of normal recombinants with their double mutant counterpart. However the absence of tetrads showing four types of cells—parental and recombinant—is unusual, and suggests that recombination occurs before chromosome replication.

Our evidence indicates: (1) unusual homothallic sexuality with 'gametes' and 'zygotes' in a wide range of sizes, DNA content and chromosome numbers; (2) complementation in 'zygotes' after the fusion of two different mutants; (3) reduction in the first one or two divisions of the complementing 'zygote'; (4) that these reduction division(s) produce either: (*a*) both parental mutant types, (*b*) recombinants, both normal and double mutant, or (*c*) apparent parental types that display unexpected behaviour. These findings suggest that the chromosomes of this organism are functionally unineme, as has been proposed[9] but that they behave unconventionally in several respects.

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Aetiology of Down's syndrome inferred by Waardenburg in 1932

IT is not widely known, and should be of historical interest, that in 1932 P. J. Waardenburg[1] suggested, in a monograph on the human eye, that Down's syndrome resulted from a chromosomal aberration due to non-disjunction. This was 5 yr after the first report of a chromosomal aberration in a mammal[2]. In 1952, Mittwoch[3] reported studying the meiotic chromosomes of a mongoloid patient and judged them to be normal. Waarden-

burg's suggestion was rarely cited, and the eventual discovery of trisomy-21 in 1959 (ref. 4) was generally received with surprise.

The relevant passage in Waardenburg's book appeared on pages 47 and 48 near the end of a chapter on mongolism. After enumerating the possible aberrations in several sentences, he went on to discuss the significance of the hypothesis for human genetics. He began as follows:

"Ich möchte die Zytologen anregen zu untersuchen ob es nicht möglich wäre, dasz hier beim Menschen ein Beispiel einer bestimmten Chromosomenaberration vorläge. . . . Man sollte einmal beim Mongolismus untersuchen ob hier vielleicht 'chromosomal deficiency' durch 'non-disjunction' oder das umgekehrte chromosomal duplication' vorliegt."

(I should like to suggest that the cytologists investigate the possibility that we have here in man an example of a specific chromosomal aberration. . . . Mongolism should be studied to determine whether there may be a' chromosomal deficiency' resulting from 'non-disjunction' or the converse, a 'chromosomal duplication.')

Waardenburg is a Dutch ophthalmologist, formerly a practitioner in Arnhem and Lecturer in Medical Genetics at the universities of Utrecht and Leiden. He is now retired in Oosterbeek.

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Electrophysiological evidence for colour channels in human pattern vision

LIGHT striking the eye is absorbed by three types of pigment preferentially sensitive to either red, green or blue light. The resulting neurophysiological signals are ultimately pooled before they activate the neural mechanism that underlies the perception of photometric brightness. On the other hand, the role of colour might be different when viewing coloured scenes containing fine detail (pattern vision) compared with standard photometric stimulus fields; colour signals may not be pooled as when perceiving brightness[1,2]. There is evidence that the human visual system handles red patterns in parallel with green and blue patterns[1,2]. I have attempted to determine spectral sensitivity curves for the red-sensitive and green-sensitive channels of human pattern vision. This was done objectively by measuring brain activity electrophysiologically.

When a subject viewed a pattern of red and black checks that exchanged places seven times per second, the visual area of the brain generated a 7 Hz alternating electrical waveform called an evoked potential (EP). Superposed on the red pattern was an unpatterned disk of desensitising light whose wavelength was λ. Evoked potential amplitude was progressively reduced by increasing the intensity of the superposed disk of light. The principle of this experiment was to compare disks of different wavelengths in their ability to attenuate EPs produced by the red pattern.

The intensity of the disk was logarithmically increased with time for 28 s and then abruptly restored to its initial value. The total intensity change was 1.9 log units. While the disk intensity was slowly changing, the red pattern, which was oscillating at 7 Hz, was continuously eliciting EPs whose moment-to-moment amplitudes were recorded as a function of time.

This was done by a Fourier analyser that operated as a narrow-band EEG filter centred on 7 Hz; its output was the running average of the EP amplitude[3]. In this way a plot of EP amplitude against disk intensity was directly obtained over the 28 s stimulus period[4]. Irregularities were reduced by electronically averaging four such samples of the required plot, allowing 20 s rest between each sampling. The technical details and experimental advantages of this new rapid technique are discussed elsewhere[4,5].

Figure 1 shows how the amplitudes of the red pattern's EPs were altered by increasing the intensity of the superposed disk of light. It should be noted that identical red stimulus patterns elicited all the EPs shown in this figure. Only the superposed unpatterned disk of light differed. Figure 1 also shows that as the desensitising disk's intensity was increased, EP amplitude was at first unaffected or even rose a little and then decreased roughly proportionally to log intensity. These two regions were separated by a 'knee' that was often clearly defined (Fig. 1 arrows). The trace marked *a* shows the noise level; it was recorded with the red pattern occluded. The evoked potential amplitude fell to noise level at an intensity close to that for which the pattern disappeared. These last findings are similar to the results of changing stimulus contrast at constant intensity[2,6–8].

Curves such as those of Fig. 1 were similar in their main features whatever the colour of the desensitising light; thus, to a first approximation, the only effect caused by changing the disk's colour was to displace the plot bodily along the intensity axis. This indicated that the EPs produced by the red pattern could, in the first instance, be treated as though the differently-coloured disks of desensitising light would have identical effects when appropriately balanced for intensity.

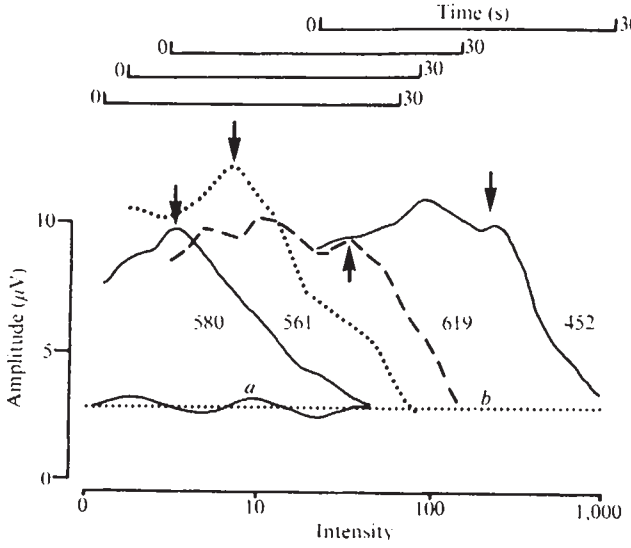


Fig. 1 Effect of desensitising light on evoked potentials elicited by a red checkerboard pattern that oscillated at 7 Hz. The 2° × 2° pattern of 676 nm, 9 min checks was viewed centrally. Ordinates: amplitude of pattern evoked potentials. Superposed on the pattern was a 6° patch of desensitising light. Intensities of desensitising light are plotted along abscissa for desensitising wavelengths of 452 nm, 561 nm, 580 nm and 619 nm. Four samples averaged for each plot. *a*, The noise level recorded with the red pattern occluded. *b*, Mean noise level. Arrows indicate positions of 'knees'. The time axes show, for each separate plot, the timecourse with which the desensitising light's intensity was changed. Half-widths of filters were 10 nm. Bipolar recordings between electrodes on the inion and 9 cm anterior along the midline. The left mastoid was grounded.

The spectral sensitivity of the red pattern EPs was found as follows: Clearly, the red pattern EPs were most sensitive to the wavelength which gave a plot at the extreme left hand side of Fig. 1. This was not, as might be supposed, when the pattern and disk had the same wavelength (676 nm); in fact the plot was shifted most to the left when the disk's wavelength was approximately 590 nm. The 590 nm curve was arbitrarily taken as reference. Some other curve (501 nm) was then shifted to the left by an amount I_{501} log units until the linear portion from the 'knee' downwards coincided with the corresponding part of the 590 nm curve. The relative sensitivity of the red pattern EPs to light of wavelength 501 nm, was then directly calculated from I_{501}. This procedure was repeated for all disk wavelengths. The filled circles in Fig. 2*a* plot relative spectral sensitivity against disk wavelength λ. Similar results were obtained by the criterion response method using evoked potential feedback[5]; these have been added in Fig. 2*a*. Peak sensitivity lies between 580–600 nm.

The range of desensitising intensities for which this spectral sensitivity curve applied was calculated as follows. When the wavelengths of the disk and of the pattern were the same (676 nm), the 'knee' was found to mark the point where the disk intensity was approximately the same as that of the pattern's bright checks (that is stimulus contrast was 33%). As mentioned above, spectral sensitivity was estimated for the sections of the plots of Fig. 1 that lay between the 'knee' and the lowest EP amplitude. Thus, the spectral sensitivity curve of Fig. 2 held from the 'knee' (where only little desensitising light had been added so that chromatic adaptation was slight) to an adapting level roughly 1.5 log units higher, where the red pattern could no longer be seen.

This use of the 'knees' illustrates how evoked potential recording can offer the possibility of quantitatively studying visual processes at everyday suprathreshold levels of sensation where available psychophysical methods may be uncertain or ineffective.

The dotted line in Fig. 2*a* shows the relative spectral sensitivity curve for luminance (brightness) perception measured by the method of heterochromatic flicker photometry. A 2°×2° unpatterned patch of wavelength 676 nm and 122 trolands was flickered against a comparison patch of wavelength λ. There was a 36°×36° white surround of 250 trolands. I have previously reported that this luminosity curve agrees closely with the spectral sensitivity curve of EPs elicited by rapidly flickering a spatially-unpatterned patch of light[9]. On the other hand, Fig. 2*a* shows that the dotted luminosity curve is quite different from the spectral sensitivity curve for red pattern EPs. The luminosity curve peaks near 555 nm. In contrast, the sensitivity of red pattern EPs is displaced markedly towards the red end of the spectrum, and peaks at 580–600 nm. This means that for pattern EPs, visual effectiveness is not directly proportional to luminance; for example Fig. 2*a* shows that red and green lights that were equally effective for the red pattern channel might differ in luminance by 0.5 log units.

Figure 2*b* shows the result of repeating these EP experiments, but with the red pattern replaced by a green (544 nm) pattern. When Fig. 2*b* and 2*a* are compared, it is clear that the green pattern's spectral sensitivity curve peaked at much shorter wavelengths (540–560 nm) than the red pattern's curve. The wavelength of the green peak was also estimated by fitting a displaced rod sensitivity curve to the data of Fig. 2*b* after allowing for prereceptor absorbtion[10]. The rod curve fitted quite well with its peak displaced to 530–540 nm. The green pattern peaked at roughly the same wavelength, or even at a lower wavelength, than the luminosity curve (Fig. 2*a*, dotted).

To what extent do the EP curves of Fig. 2 represent the action spectra of red-sensitive and green-sensitive channels in pattern vision? Isolation of colour channels

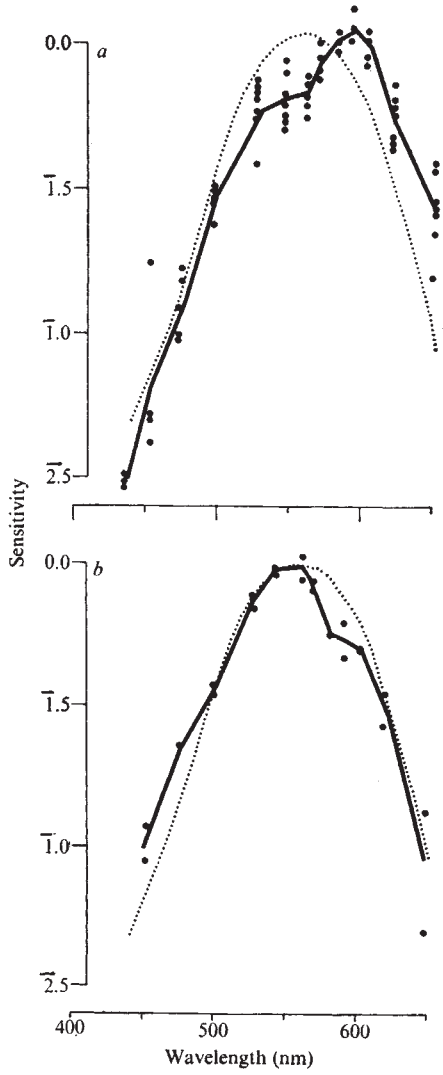


Fig. 2 *a*, ——, The relative spectral sensitivity for brain potentials evoked by a red (676 nm) pattern; ●, individual points; . . . ., relative luminosities of spectral lights determined by heterochromatic flicker photometry. *b*, As for *a* except that the pattern was green (544 nm).

is notoriously difficult, as the action spectra of different photopigments overlap. Thus, even if each colour channel subserving pattern vision were fed exclusively by a single type of photoreceptor, then even monochromatic lights would stimulate all channels to some extent. In the present case the blue-sensitive pigment was most probably unimportant, not only because its spectral sensitivity is well separated from the red and green-sensitive pigments, but also because a fine stimulus pattern was used (9 min checks). It is well known that the spatial resolving power of the blue-sensitive visual mechanism is coarse[11]. The shapes of the spectral sensitivity curves were consistent with some minor contribution from green receptors in Fig. 2*a* and some minor contribution from red receptors in Fig. 2*b* Furthermore, the EP curves of Fig. 2 peak at wavelengths that agree with estimates for the peak sensitivities of the red and green photopigments[12,13] or colour mechanisms[14–19]. All this indicates that Fig. 2*b* to a fair extent represents the action spectrum of the green photopigment and that Fig. 2*a* describes, though with rather less precision, that of the red pigment.

The data reported above extend the findings of previous experiments in which subjects viewed an equiluminant pattern of adjacent red and green checks (or bars). Large pattern-specific EPs were recorded each time that the

red and green checks (or bars) exchanged places[2,7,20,21]. I pointed out that such EPs could not exist unless red and green signals were still to some extent segregated on arrival at pattern-sensitive neurones[2,7]. Furthermore, chromatic segregation might even be complete[21]. This explanation would also be consistent with the McCollough effect[1] and with evidence that lateral interaction occurs within each class of cone[22–24]. It is important, however, to distinguish this suggestion from the colour segregation observed when monochromatic patterns stimulate the chromatically-adapted eye (for example, as above and in ref. 22). The latter conditions would reveal peripherally-determined red, green and blue colour channels even if colour signals were no longer segregated when they reached pattern-sensitive neurones.

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Prehistoric human activity and blanket peat initiation on Exmoor

It now seems that the shallow tracts of peat which are so abundant in western parts of the British Isles began their development at about the time when the first signs of human activity became evident in the pollen record[1,2] contained in the peat. These blanket mire complexes or "Terrainbedeckende Moore"[3] have received relatively little attention from palaeoecologists and radiocarbon-dated pollen profiles are few; those which exist refer mainly to deposits from Ireland[4,5] and the Southern Pennines[6,7]. We now present a radiocarbon-dated pollen diagram, from a blanket mire on Exmoor, Somerset, which throws further light on the coincidence of prehistoric human activity and the initiation of blanket peat formation.

The deepest blanket mires found on Exmoor were on an upland plateau site termed The Chains. A monolith of peat from the mire was excavated and this was used both for palynological work and for radiocarbon dating. Pretreatment of the samples for dating was carried out according to the specification of the Palaeoecology Laboratory, Belfast, where the dating was performed. In effect this pretreatment involved the extraction of the fine particulate matter from the peat by a process of KOH digestion followed by sieving. The dated material thus consisted largely of pollen and spores together with fine fragments of fungal origin. Contaminant root material penetrating the profile from above was thus eliminated.

Selected results of pollen analysis together with the radiocarbon dates are shown in Figs 1 and 2. The diagram has been zoned on the basis of the characteristics of the pollen assemblage (see Table 1); in Table 1 the probable corresponding archaeological or historical period is also suggested.

The diagram bears a strong similarity to those published from mid-Wales[8] and the Southern Pennines[7]. The dating of the basal layers at about the time of the arrival of farming cultures lends further support to the suggestion that the introduction of grazing animals[2] or the ploughing of marginal land[9] may have assisted in the initiation of these deposits.

Shallow blanket peats of about 1 m in depth are more

Table 1 The Chains pollen zones and their interpretation

Pollen Zone	Major characteristics	Interpretation and date (radiocarbon yr)
EC1	Relatively high *Ulmus* and *Pinus*	Undisturbed woodland before arrival of farming cultures
EC2	Decline in *Pinus* and *Ulmus*. Increase in *Alnus* at the expense of *Quercus*. Increase in *Pteridium; Plantago lanceolata* is consistently present	Arrival of farming cultures and the response of the woodland to their activities. Extrapolation from dates, assuming an even rate of peat formation suggests the period covers about 5,000–3,800 b.p. (Neolithic)
EC3	Gradual recovery in *Ulmus* to a maximum at end of zone. No recovery in *Pinus*. *Plantago* sporadic and *Pteridium* erratic	An unsettled period with no permanent human settlements. Dates between about 3,800 and 2,300 b.p. (Bronze Age)
EC4	Abrupt decline in *Ulmus* and increase in *Betula*, *Pteridium* and *Plantago*. A decline in *Tilia* precedes that of *Ulmus*	Increased activity in terms of farming, woodland clearance and settlement. Dates, about 2,300–1,500 b.p. (Iron Age–Roman)
EC5	A sudden decline in *Pteridium* and *Plantago*. An increase in *Betula*	Reduced human activity. Period begins at about 1,500 b.p. (end of Roman), but is of uncertain duration
EC6	Gradual rise in *Pteridium* and *Plantago* to a maximum at the end of the zone. Decrease in *Alnus*, rise in *Ulmus* and *Pinus*	Increased settlement and deforestation, especially of lowlands and valleys. Uncertain date, but possibly culminating in the Napoleonic Wars, about 150 yr ago
EC7	Decrease in *Pteridium* and *Plantago*. *Pinus* reaches maximum	Reduced intensity of farming. Possibly past 150 yr

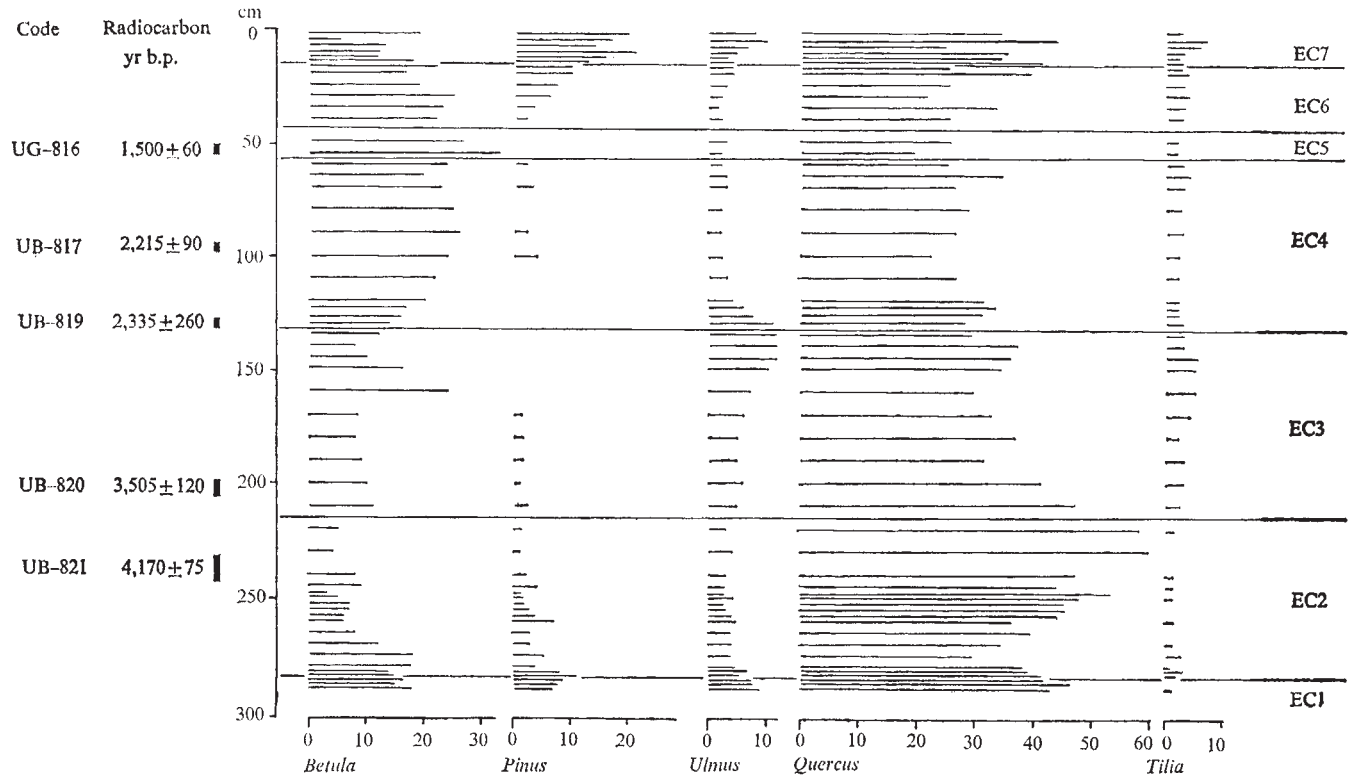


Fig. 1 Selected pollen data and radiocarbon dates from the peat profile at The Chains, Exmoor. (% Arboreal pollen)

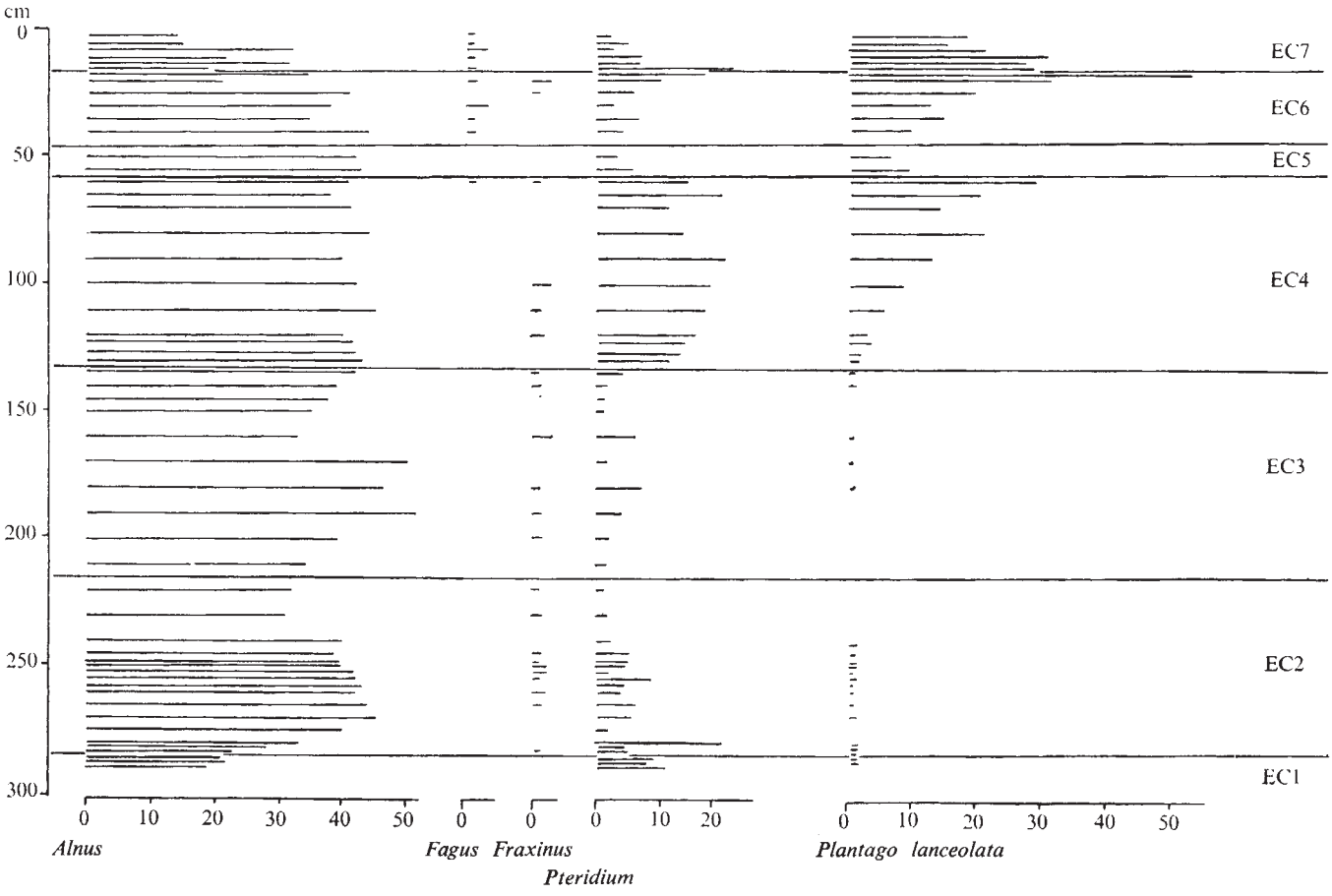


Fig. 2 Further pollen data from The Chains, Exmoor.

matters arising

Singing muscles in a katydid

SIR,—Your Insect Physiology Correspondent[1] has perpetrated a common error by stating that the tymbal muscles of cicadas may contract at 4,500 Hz. The song of cicadas, like other types of insect, consists of pulses of sound. The sound frequency, which is controlled by resonance of the abdominal air cavities, was measured by me as 4,500 Hz in some large cicadas in Ceylon, but is considerably higher in small species. The pulse modulation frequency, which may relate to the contraction frequency of the tymbal muscles, has never been observed higher than about 600 Hz, even in species with a myogenic contractile mechanism.

Neither your correspondent, nor Josephson in the article quoted[2] mention that high contraction frequencies in neurogenic muscles have been measured not only in insects but also in some vertebrate muscles producing only a small output of energy (the crichothyroid muscle of the bat[3]; and a fish swim-bladder muscle[4]). In all cases in fast neurogenic muscles, there is a correlated development of the sarcoplasmic reticulum involved in calcium transport.

Yours faithfully,

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[1] *Nature*, **248**, 106 (1974).
[2] Josephson, R. K., *J. exp. Biol.*, **59**, 781 (1973).
[3] Revel, J. P., *J. Cell Biol.*, **12**, 571 (1962).
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Biosynthesis of bacteriochlorophyll

SIR,—Bacteriochlorophyll (BChl) production in nonsulphur purple photosynthetic bacteria growing anaerobically is inversely related to light intensity, and introduction of O_2 causes a rapid suppression of pigment synthesis. In a recent report[1], Davies *et al.* summarise findings which lead them to conclude "the original suggestion of Cohen-Bazire *et al.*[2] that the synthesis of BChl is controlled by the redox state of one or more of the components of the electron transport chain, is correct."

In this connection, Davies *et al.* cite experiments[3] with intact cells in which various respiratory electron transport inhibitors were found to interfere with the synthesis of magnesium protoporphyrin. Since interruption of electron transport in intact cells can be expected to have numerous ramifying effects, there is reason to question the statement that "one or more of these [electron transport] components has been shown to be directly involved in the insertion of magnesium into protophorphyrin".

Davies *et al.*[1] suggest that "direct" control of BChl synthesis by O_2 (and light intensity) is also effected through regulation of aminolaevulinate (ALA) synthetase, the first enzyme of tetrapyrrole biosynthesis. They assert that ALA synthetase may be regulated by a trisulphide of glutathione or cystine which, in its oxidised form, increases activity of the enzyme. According to their scheme, oxygen (or light) has the effect of oxidising carriers of the electron transport chain, which in turn cause oxidation of an unidentified sulphydryl compound to its disulphide form; the latter than is presumed to oxidise the "reduced polysulphide activator" to its active (stimulatory) state. On this basis, O_2 might be expected to accelerate BChl synthesis, rather than inhibit, but Davies *et al.*[1] seem to suggest that O_2 also disturbs sulphur metabolism so as to interfere with biosynthesis of the trisulphide activator. Elsewhere[4], Neuberger *et al.* express the view that "oxygenation may lead to direct inactivation of the activator". We have been unable to rationalise the regulation of BChl synthesis by oxygen in terms of their interpretations.

Our recent experiments[5] with mutants of *Rhodopseudomonas capsulata*, blocked at different points in respiratory electron transport, demonstrate that the rapid inhibition of BChl synthesis by oxygen is independent of the cell's metabolic capacity to use O_2 as a terminal oxidant or to deliver reducing equivalents to the aerobic electron transport system. This argues cogently against the "redox carrier governor" postulation for regulation of BChl synthesis, and we have proposed an alternative working hypothesis in the study cited[5].

Yours faithfully,

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[1] Davies, R. C., Gorchein, A., Neuberger, A., Sandy, J. D., and Tait, G. H., *Nature*, **245**, 15 (1973).
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[4] Neuberger, A., Sandy, J. D., and Tait, G. H., *Biochem. J.*, **136**, 491 (1973).
[5] Marrs, B., and Gest, H., *J. Bact.*, **114**, 1052 (1973).

DR NEUBERGER REPLIES: At the time when the paper by Davies *et al.*[1] was first submitted to *Nature*, all the known facts discovered by others and ourselves were compatible with the proposition first put forward by Cohen-Bazire *et al.* in 1957 that the electron transport chain in some way exerted a direct control over bacterial chlorophyll synthesis. Later experiments by Marrs and Gest published in 1973[2] and our own work published later in that year[3] indicated that the effects of oxygen may be more specific and may be exerted through its action on sulphur metabolism, and in particular may be due to the depletion of the cellular content of a trisulphide activator of the enzyme. In this respect we are in

essential agreement with Gest and Marrs and our current work also is in accord with this general idea. It is, however, still possible that the activation process, which was first described by Marriott *et al.*[4], involves in some way the electron transport chain. The possibility also exists that similar mechanisms operate *in vivo*, but data at present available do not allow a definite conclusion to be reached.

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[1] Davies, R. C., Gorchein, A., Neuberger, A., Sandy, J. D., and Tait, G. H., *Nature*, **245**, 15 (1973).
[2] Marrs, B., and Gest, H., *J. Bact.*, **114**, 1052 (1973).
[3] Neuberger, A., Sandy, J. D., Tait, G. H., *Biochem. J.*, **136**, 491 (1973).
[4] Marriott, J., Neuberger, A., and Tait, G. H., *Biochem. J.*, **111**, 385–394 (1969).

Microstructure of magnesium oxychloride cements

SIR,—Matkovic and Young in their article on the microstructure of magnesium oxychloride cements[1] reported some interesting observations, and raised some points for discussion. I would like to discuss those points which have wider implications.

The authors coated their samples with a Au–Pd layer of unstated thickness before examination in a scanning electron microscope (SEM). In the case of the metal coated specimen, SEM only reveals the outer topography of the metal layer. Figure 1 shows a section through three parallel needles each 2,000 Å thick, separated by 500 Å. The volume porosity of this packing is 37%. Assuming that the depositing vapour had a uniform density, any layer more than 250 Å (that is $\frac{1}{8}$ of the needle diameter) thick, will start to coalesce them. If the film thickness is 500 Å ($\frac{1}{4}$ of the needle diameter) the shaded areas of Fig. 1 will receive double the amount of metal necessary to fill them up. This excess metal will pile up locally, making the outer surface slightly undulated (indicated by the broken lines), and will be difficult to resolve into three. A thicker coating will make them appear monolithic. With lower porosity, a lower film thickness will be necessary to submerge them. If the needles are diverging, a metal film of proper thickness will make them appear to intergrow at the points of nearest approach, though in reality they do not. It can be seen that unless the coating thickness is adjusted to take care of particle size and density

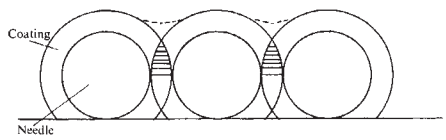


Fig. 1 A section through three parallel needles, each 2,000 Å thick, separated from one another by 500 Å. The broken lines indicate the slightly undulating outer surface formed by excess metal.

of packing in the specimen, quite a misleading topography may result.

Figure 1*c* of ref. 1 shows that the average apparent diameter of the crystals is about 1,200 Å. For the real diameter, twice the film thickness has to be deducted from that figure. For crystals of 1,200 Å real diameter, a film thickness of 150 Å or less will be required before it can be distinguished whether they are intergrowing or not. The above crystals were, however, the initial reaction products. The subsequent products are expected to be smaller and more densely packed. It is perhaps not surprising that the authors found that in older samples, the crystals were intergrown and the samples had a monolithic appearance. The smoothness of the cracked surfaces in their Fig. 3 can also be explained on this basis, though there are other alternatives.

The term 'intergrowth' has been used rather uncritically. There are two types of crystal–crystal intergrowth. A crystal may intergrow into another crystal without having any structural continuity, like a nail in a piece of wood. This is a special case of mechanical intergrowth. The second type of intergrowth involves structural continuity between the participating crystals. For this type of intergrowth to occur the crystals need to meet each other in definite orientations, satisfying the laws of twinning. Matkovic and Young seem to imply the second type of intergrowth. I wonder if they have tried, on this basis, an order of magnitude calculation on the probability of formation of a three dimensional load bearing structure. The result of that calculation would be quite illuminating.

Note added in proof: Re-examination of Fig. 1*c* reveals isolated needles whose real diameters are equal to the stated film thickness. How many more of them were in the real sample?

Yours faithfully,
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[1] Matkovic, B., and Young, J. F., *Nature phys. Sci.*, **246**, 79 (1973).

DRS YOUNG AND MATKOVIC REPLY: It is certainly true that one of the problems of SEM is the need to have a conducting layer on the surface of inorganic materials. We are aware of the inherent problems of metal coatings, but do not think it invalidates the results presented in the original article. We used comparatively light coatings (~250 Å) of gold–palladium, and comparisons of such coatings with pure carbon coatings (which are transparent to secondary electrons) on samples of portland cement compounds, have not shown substantial differences in microstructural details. We therefore have confidence in the qualitative observation described in our article.

The term 'intergrowth' has been deliberately used in a vague sense and we regret any resultant misinterpretations. On the basis of SEM observations alone it is not possible to draw any definite conclusions concerning the exact interactions between crystals. We feel that, in order to explain the high mechanical strengths, these interactions must be stronger than weak Van der Waals surface forces, but may range from strong 'solid–solid contacts' postulated for hydrated portland cements[1], to true structural continuity.

Finally, we emphasise that these results are preliminary observations. We advanced our ideas in the interests of suggesting an alternative approach to the problem of strength development in cementitious systems. We realise that SEM cannot provide all the answers and we feel that, at present, attempts to make quantitative predictions may be premature.

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[1] Feldman, R. F., and Sereda, P. J., *Proc. fifth int. Symp. chem. Cements, Tokyo, 1968*, **III**, 36–44 (Cement Association of Japan, Tokyo, 1969).

Photosynthesis in leaves exposed to SO_2 and NO_2

SIR,—Bell and Clough[1] have reported that the growth of S23 ryegrass was substantially reduced by exposure to 12 p.p.hm. (parts per hundred million) SO . Bleasdale[2] grew the same variety of ryegrass in greenhouses ventilated with polluted air from out-of-doors on a suburban site in Manchester and compared the growth with that in a greenhouse ventilated with purified air. There were reductions in the dry weight of the plants amounting to 57% in the polluted atmosphere, even though the SO_2 concentrations were below 3 p.p. hm. for 39 out of 42 d and the daily

means did not rise above 9 p.p.hm. at any time.

Bleasdale's data appear to show that S23 ryegrass is more sensitive to SO_2 than is apparent from the study by Bell and Clough. This discrepancy may be the result of their having performed experiments in different conditions, but it may equally have resulted from Bell and Clough's simulated SO_2 pollution rather than polluted air from outside. In Bleasdale's experiments other air pollutants would also have been present. One such is NO_2. This is found in polluted atmospheres because NO is produced by combination when fossil fuels are burnt, and is quickly oxidised in air to NO_2. We have recently completed some experiments in which the combined effects of SO_2 and NO_2 pollution on photosynthesis in the pea,

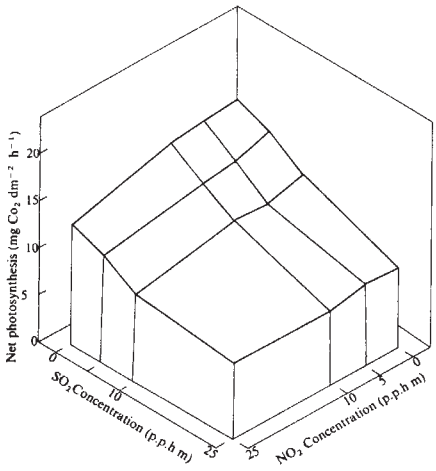


Fig. 1 Effects of simulated SO_2 and NO_2 pollution on net photosynthesis in *Pisum sativum*. Fumigation conditions: 1.20 air changes min^{-1}; temperature, 21° C; light intensity, 73 J^{-2} s^{-1}; water vapour pressure deficit, 995 N m^{-2}.

Pisum sativum, were examined over a concentration range of 0–25 p.p.hm. for each gas. Initially, exposure to these pollutants increased net photosynthesis, but the effect was short lived and a substantial inhibition soon followed. The magnitude of this inhibition for 15 different treatments is shown in Fig. 1, with the control included for comparison. Analysis of this factorial experiment revealed statistically significant effects of SO_2 and NO_2 ($P<0.001$ in both cases) but no significant $SO_2 \times NO_2$ interaction. It is clear, however, that the effects of the two pollutants are at least additive. These observations were made on detached leaves and no visible injury accompanied the depression of net photosynthesis, but more prolonged exposures to concentrations of 10 p.p.hm. and above of both SO_2 and NO_2 were found to produce visible lesions on intact plants. Visible injury to crop plants after simultaneous exposure to SO_2 and NO_2 has been reported previously[3].

Our data suggest that combined effects of SO_2 and NO_2 might explain the growth inhibition caused by urban pollution[2], and that this possibility should be considered in future studies.

J. N. Bull acknowledges the receipt of a CASE award from the Science Research Council, and we are grateful to ICI Ltd for financial support and Mr J. F. Newman for his advice.

Yours faithfully,

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1 Bell, J. N. B., and Clough, W. S., *Nature*, **241**, 47–49 (1973).
2 Bleasdale, J. K. A., *Environ. Pollut.*, **5**, 275–285 (1973).
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Regulation of albumin-bound tryptophan

Sir—Madras *et al.*[1] recently reported on the effect of tryptophan concentrations in serum and brain concerning its conversion to serotonin in rats. This and related work have been summarised by Fernstrom and Wurtman[2] who suggest that albumin-bound tryptophan is the precursor of serotonin. These authors[1,2] have found that decreased nonesterified fatty acid content (albumin-bound) in the serum caused by administration of carbohydrate or insulin results in higher concentrations of albumin-bound tryptophan, while fasting (high fatty acid content in serum) results in lower albumin-bound tryptophan levels, suggesting that fatty acids competitively prevent the binding of tryptophan by albumin.

If one assumes a serum albumin concentration of 4 g per 100 ml, the data of Madras *et al.*[1], on the concentrations of serum-bound tryptophan and fatty acids can be converted into moles of tryptophan or fatty acid bound moles of serum albumin (Table 1). Thus conditions which decrease concentrations of fatty acids in the serum increase concentrations of albumin-bound tryptophan. McMenamy and Oncley[3] found that bovine albumin bound 1 mol of L-tryptophan per mol of albumin. Addition of fatty acid decreased the binding capacity of the albumin for tryptophan by 0.1 mol for each mol of fatty acid. When 2.0 mol of oleate was present the albumin bound 0.75 mol of tryptophan.

Since 2.0 mol of fatty acid per mol of albumin represents the largest fatty acid ratio in Table 1, I assume that a minimum of 0.75 mol of tryptophan could be bound to the albumin rather than the maximum of 0.135 mol observed if fatty acid competition were the limiting factor.

The free energy (−7.2), enthalpy (−14.5) and entropy (−24) changes determined by Fairclough and Fruton[4] for the binding of L-tryptophan to serum albumin are similar to those observed for binding of iodide[5], lysolecithin[6] and probably dodecyl sulphate[5] to the high energy site of albumin. Thus such a material, which is strongly bound to the albumin, might decrease the amount of this site available for tryptophan and so function, along with fatty acids, to regulate the amount of tryptophan bound.

Yours faithfully,

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2 Fernstrom, J. D., and Wurtman, R. J., *Sci. Amer.*, **230**, No. 2, 84–91 (1974).
3 McMenamy, R. H., and Oncley, J. L., *J. biol. Chem.*, **233**, 1436–1447 (1958).
4 Fairclough, G. F., jun., and Fruton, J. S., *Biochemistry*, **5**, 673–683 (1966).
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Table 1 Effects of glucose and diet on bound tryptophan and fatty acids

	Effect of glucose			Effect of diet		
	Control	Glucose 1 h	Glucose 2 h	Fasted control	Carbohydrate + fat	Carbohydrate
Serum bound tryptophan mol per mol albumin	0.091	0.13	0.135	0.09	0.11	0.135
Non-esterified fatty acids mol per mol albumin	2.0	1.14	1.05	1.46	1.08	0.53

book reviews

De Broglie's philosophy

Louis de Broglie: Sa conception du monde physique, By A. George, *et al.* Pp. xxviii+387. (Gauthier — Villars: Paris, 1973.)

LOUIS DE BROGLIE'S 80th birthday (August 15, 1972) came close enough to the 50th anniversary of his three famous notes to *Comptes Rendus* (September–October 1923) and the publication of his doctoral thesis (November 25, 1924) to warrant a double celebration. This collection of articles by his close colleagues, old and young, describes both his long career as a teacher and leader of research and the contributions that he and his pupils have made to "the past and the future of wave mechanics". Handsomely produced, elegantly phrased, and well integrated despite more than twenty different authors, it will prove a valuable source for biographers and for historians of science.

The accuracy of the personal picture cannot be easily estimated in a work of this kind, whose whole emphasis is naturally on the positive and virtuous —the guide, philosopher and friend, the lucid and eloquent teacher, the imaginative scientific intellect, still enthusiastic in old age, still able to state, more clearly than his pupils, the essential stages in his own scientific development and his own analysis of current problems. No man is perfect; but we do well to admire these qualities of greatness.

But the scientific work, being in the public domain, must be appraised more judiciously. Its standing depends not on the fact that it was done by M. de Broglie, but that it conforms to reality and is accepted by other competent scientific experts. This is the harsh law of science which defers not to prince, professor or poet.

Of the thesis of 1924 there can only be the highest praise. The very idea of 'matter waves' is the most revolutionary innovation in the philosophy of nature since the ancient Greeks. This significant step in modern physics was no mere stumble into the unknown, but a tremendous leap, gracefully poised on the classical principles of Fermat and Maupertuis but vaulting beyond Einstein's conjecture on the particulate nature of radiation. The first half of this book deals with the theoretical background of this brilliant paper, and on some of its modern consequences, in electron microscopy, and so on.

So profound, indeed, was the revolution of thought that the revolutionary has spent the rest of his life trying to resist the further, yet more radical, statistical interpretation of the wave function. The papers in the second half of this volume record the considerable efforts of de Broglie and his school to reconcile waves with particles in a more deterministic scheme. Since this work is largely ignored by other theoretical physicists, one must ask why it has not found wide acceptance.

Nobody who has seriously grappled with (let alone had to teach!) the modern paradigm of quantum theory can feel completely satisfied with its present form. The essential localisation of the particle event is not intuitively consistent with the equally certain evidence of interference between extended wave fields. The fantastically precise discreteness of the rest mass and charge of an electron is a fact of nature that is difficult to swallow philosophically. The operator formalism of Heisenberg and Dirac seems to answer all the practical questions but leaves hollow the interior reality. The extraordinary paradox of a physical quantity that seems to depend upon the state of mind of the physicist is not to be shrugged off without comment.

There is, of course, a vast literature on these problems, to which the de Broglie school make almost no reference. Their theme is the ultimate 'reality' (potentially unequivocal observability?) of wave fields, of which the particles are singularities and by which they are guided. In his work of the 1930s, de Broglie concentrated on a theory of photons, which were assigned a very small rest mass to make them localisable. In the 1950s, he developed the 'double solution' for the particle singularity and its guiding wave, explaining the statistical phenomena of particle physics as manifestations of a hidden thermodynamic ensemble. Are these models of submicroscopic physics convincing, or worthy of further sustained effort?

Despite many elegant results, the physical systems to which they are applied are very simple (for example a few nearly free electrons) and no new phenomena are predicted for experimental verification. Indeed, it is taken for granted (although with rather meagre proof) that the whole scheme must be capable of reproducing the results obtained by conventional methods for almost all atomic, nuclear and high energy systems. The de Broglie model is scarcely falsifiable, since it is presented as an alternative 'explanation' of quantum physics at the deepest level, incorporating automatically all existing theoretical achievements such as quantum electrodynamics, many body theory, and broken symmetry schemes.

The necessity for such an explanatory scheme thus rests on metaphysical rather than on physical considerations. The practical physicist may continue to solve all his problems by the conventional operator formalism, and need not learn the more complicated mathematical techniques of non-linear field theory. Until experiment, or some astonishing theoretical coup, demonstrates the practical superiority of the de Broglie interpretation, we can safely ignore it.

Does that explain, or justify, the neglect of this work by almost all orthodox physicists? It would be more honest to say that despite all the mathematical effort that has been lavished on it, the whole project rests upon too narrow a foundation of hypothesis and technique. The natural phenomena are richer (for example neutrinos, muons, pions, strong and weak forces) and our mathematical resources more diverse (finite and continuous groups, topology, tensor calculus and so on) than this book seems to imagine. For aesthetic reasons, and in modest deference to the unknown knowledge of future men, we do well to doubt that physics is conceivable only in the manner inherited from Newton, Laplace and Einstein. I would rather live uncomfortably with the paradoxes of quantum physics, hoping that some day they will be resolved, than accept, for the sake of peace of mind, a well meant but essentially backward looking scheme which promises more than it has yet achieved.

JOHN ZIMAN

Fine structure of algae

The Fine Structure of Algal Cells. By John D. Dodge. Pp. xii+261. (Academic: London and New York, 1973.) £6.

THIS book is well produced and many of the illustrations will doubtless be

turned into lantern slides. The text, on the other hand, reads like a catalogue, with all the disadvantages of this type of format. Moreover there is no flicker of interpretative insight directed towards a reader who may not spontaneously appreciate the scientific interest of the phenomena or structures described.

This is not all the result of compression to reduce costs since, minor errors or omissions apart, some hazards to potential users have been deliberately inserted by the author or publisher without effecting great savings. Thus all too many micrographs are labelled with a generic name only, as if species of algae do not matter. One picture of scales is even labelled *"Chrysochromulina?"*. Perhaps some tyro may value this and memorise it as an examination gimmick. An older reader, on the other hand, perhaps a non-botanist hoping to enter the fine structure field personally, must learn at the outset that, especially among algae, species may matter very much and carelessness here can invalidate or render unrepeatable the most elaborate exercises of other kinds. Any book which suggests, even by its format, that this is not so is being less than helpful to a serious and desirable type of reader.

Further, the omission of titles from the papers listed at the end, removes at a stroke the future usefulness of the book as a work of reference while greatly increasing the difficulty of effective proof reading. Thus on page 166 an important topic is said to have recently been summarised by an author dated 1972e while in the literature list one finds only 1972f (subject unknown) following 1972d (subject also unknown).

While, therefore, this book will be useful for a limited time and purpose for which it ought to be bought by libraries, it is not one which I would give as a present to a friend, colleague or student. Considering the intrinsic interest and beauty of the subject this is disappointing. I. MANTON

The first clover leaves

The Primary Structure of Transfer RNA. By T. V. Venkstern. Translated from Russian by Basil Haigh. Pp. x+296. (Plenum: New York and London, 1973.) $29.

THIS monograph sets out to cover the entire field of transfer RNA primary structure in textbook form. It is an extended edition of a Russian text first published in 1968 and in this revised version surveys the literature up to July 1971. Each of the 22 transfer RNAs which had been sequenced by that time are described in considerable detail. The constant features of transfer RNA architecture are also discussed, together with the evidence supporting the clover leaf hypothesis of the base-pairing of transfer RNA sequences. Little serious attempt is, however, made to relate features of individual sequences to the functional centres which determine the acceptor or adaptor roles of transfer RNA, other than in a consideration of the codon recognition properties of different anticodon loop structures. Hence those wishing to comprehend the structure–function relationship of transfer RNA will not find in this monograph a topical account of how the nucleotide sequence of a transfer RNA molecule can determine its function. The three-dimensional structure of transfer RNA obtained from X-ray crystallography is now clearly on the horizon and it should soon be possible to provide a more meaningful topological interpretation of the significance of those regions of transfer RNA sequences which lie outside the anticodon.

Much of this book is given over to a clear and detailed account of how the first few transfer RNA sequences were determined. The author takes us back to the initial attempts to determine nucleic acid primary structures and enables us to re-live that exciting period when the first few transfer RNAs were being sequenced. A considerable proportion of the narrative describes methods for the sequencing of non-radioactive RNA and many of these procedures have been largely supplanted by newer methods of sequence analysis which greatly reduce the work load of the experimentalist. There is little more than passing reference to the methods developed in Sanger's laboratory for sequencing ^{32}P-labelled RNA, even though most transfer RNA sequences are now derived using these techniques. Furthermore several other publications give a much more comprehensive and useful description of nucleic acid sequencing.

The chapter of this book which discusses the minor nucleotides of transfer RNA is by far the most topical and presents a thorough account of the physicochemical properties of these components. In view of the elaborate system of enzymes which exists for modifying transfer RNA it is hard to conceive that the modifications do not have an important biological function. Nevertheless the more inquisitive reader would probably wish to see expanded the sections of this book which describe the roles of the different isoacceptor transfer RNAs and the functions of the different minor components of transfer RNA.

As a result of the considerable effort now being expended in determining nucleic acid structures it is clearly almost impossible for any book on transfer RNA to be up to date by the time that it is published. It has taken three years for the English version of this book to appear and during that time the number of sequenced transfer RNAs has increased from 22 to nearly 70. A complete summary of all known transfer RNA structures (*The Handbook of Nucleic Acid Sequences*, by B. G. Barrell and B. F. C. Clark) has recently been published as a loose-leaved folder by Joynson-Bruvver, Oxford. This is to be annually updated and will be rather more realistically priced. T. V. Venkstern's book is more of value as a comprehensive account of the beginnings of nucleic acid sequencing.

P. W. PIPER

Evolution of plants

Chromosome Botany and the Origins of Cultivated Plants. By C. D. Darlington. Third (revised) edition. Pp. xvii+237. (Allen and Unwin: London, September 1973.) £4.40.

THE fascination of plant evolution is the great variety of mechanisms that have been involved. Plant species have certainly not plodded dully through time, losing a few genes here, gaining a few genes there: they have flashed into polyploidy, chromosome structural changes, inbreeding, apomixis, hybridity, with almost breathtaking frequency. The plant species which have stuck to an ordinary outbreeding diploid type of evolution are probably fewer than those which have not.

Since so much of this cunning and opportunism is tied up with chromosomal change, a book on chromosomes in plant evolution, by the cytologist to whom much of our understanding of chromosomes is due, is surely to be welcomed and respected, especially since it now reaches the eminence of a third edition. The book is also important because it is one of the very few in which the origins of cultivated plants are discussed, making use of recent evidence.

Alas for high hopes. The trouble is that the author falls a victim of his own enthusiasm. One is given too great an impression that plant evolution can be explained solely in terms of chromosomes: changes at the level of the gene cannot be disregarded. One is given a fascinating picture, but one which is capricious and worrying, and sometimes difficult to understand.

Yet the book contains the distillation of a lifetime: a provocative variety of evidence and conclusions on plant evolution that is unique, and a collection of information on crop plant evolution that is not available elsewhere. This is the reward for the reader.

A. D. BRADSHAW

Sad time for salmon

International Atlantic Salmon Symposium. Edited by Morden W. Smith and Wilfred M. Carter. Pp. xi+504. (Atlantic Salmon Research Trust: Fanham, Surrey; International Atlantic Salmon Foundation; New Brunswick, 1973.) £6.50 boards; £5 paper.

THIS collection of papers is the published proceedings of the "Symposium on the Atlantic Salmon: the Management, Biology and Survival of the Species", held at the Huntsman Marine Laboratory, St Andrews, New Brunswick, in September 1972. It consists of thirty-one major contributions under six main headings; the effects of man and the changing environment, physiology, ecology, aquaculture, environmental engineering and fishery economics, and conservation and fisheries.

With such a wide-ranging approach to the problems which surround the Atlantic salmon it is not surprising that several of the papers seem to have little direct relevance to the main objects of the symposium. One contribution invokes the aid of systems analysis for salmon conservation in a dauntingly titled paper, "the FASTRR concept (Facility for Atlantic Salmon Technological Research and Restoration). A status report". This concludes "the design and construction of an improved intensive culture hatchery depends on the determination of biological and physical needs, their estimated implementation and operating costs as balanced against predicted benefit to the overall project objectives. Elements of this study hopefully will ease the decision maker's task of quantitative initial assessment, choosing between alternates, and managing efficiently . . .". (One wonders what Frank Buckland, Inspector of Salmon Fisheries, 1867-1879, who managed to hatch salmon and trout eggs in the kitchen of his home in Albany Street, London, would have said of that?)

The salmon, in an unique way, is the focus of emotional attitudes. As an economic, leisure, or food resource it is probably without equal, but its decline in the industrialised and heavily populated North Atlantic countries has been quite dramatic. Reaction to dwindling salmon stocks in many countries suggests that although this may be more or less acceptable, it is clearly not acceptable for other nations to exploit the stocks outwith the national boundaries. Thus, the relatively recently developed high-seas fisheries conducted off the coast of West Greenland, and also in the Norwegian Sea, has resulted in a crisis within the salmon-producing countries, made still more emotional by the fact that the country mainly operating the fishery is not a salmon producer. This development has placed the conservation of the Atlantic salmon firmly in the field of international relations, and as Dr Wilfrid M. Carter, co-editor of this volume, remarks, "the Atlantic salmon, perhaps more than any other fish has become symptomatic of the unwillingness of the international community to face and to solve critical problems . . .".

The contributions in this volume essentially fall into three categories (although they are not so divided by the organisers): the biological papers, some of which have direct relevance to the conservation of the species, papers on the management of stocks, and those directly related to the international management of the species. This last category would seem to have the most direct bearing on the major problem confronting the salmon. In this context, the review by B. B. Parrish of the work on the ICES/ICNAF Joint Working Party on the North Atlantic Salmon is a major contribution, summarising the problems involved and distinguishing the areas of biological knowledge that require to be clarified before more precise assessments of their effects can be made. But as D. L. McKernan points out elsewhere in the volume, the adoption of an ICNAF formula to phase out the non-native high seas fishery off Greenland, and to impose a catch limit on the inshore fishery, offers reason to be cautiously optimistic for the future of the Atlantic salmon. ALWYNE WHEELER

Weather forecast

Radar Observation of the Atmosphere. By L. J. Battan. Pp. x+324. (University of Chicago: Chicago and London, 1973.) £7.15.

THERE has been a considerable increase during the past two decades in the use of electronic techniques for the remote sensing of the atmosphere, with radar playing an important if not dominant part. So much new material had become available that the author has carried out an extensive revision of his earlier work *Radar Meteorology*, first published in 1959. The present volume under its new title is solely concerned with the application of microwave radar in the study of atmospheric phenomena including precipitation, cloud, wind and clear air turbulence. The author has succeeded in covering most of the relevant material in less than 300 pages of text. This should have considerable appeal to a wide scientific readership apart from meteorologists, particularly those who lack a specific background in electronics.

The principles of radar are well illustrated although the author's very brief introduction concerning the properties of electromagnetic waves will hardly benefit a reader who is not already familiar with the subject. The remainder of the book deals with radar applications and the related atmospheric physics in appreciable depth. Although space may have been limited it would have been attractive if the author had covered in the same manner some aspects of upper atmospheric work including chaff and meteor radar as well as incoherent scatter techniques. Numerous technical details of radar installations are given in an appendix and readers will welcome a very comprehensive and up to date list of references. H. G. MULLER

Strange coincidence

The Challenge of Chance: Experiments and Speculations. By Alister Hardy, Robert Harvie and Arthur Koestler. Pp. 280. (Hutchinson: London, November 1973.) £3.

DURING a recent visit to America I was met at Roanoke Airport by my friend the statistician, I. J. Good, of the Virginia Polytechnic. Good excitedly asked me what type of plane I had flown in on and when I told him a Boeing 727 he cried " I knew it. I knew it". He then pointed to the licence plate of his Ford Mustang, which boldy featured the inscription "CRE 727". Permanent testimony to the happy correspondence between my initials, the plane I had flown in and Jack Good's car exists in the form of a licence plate which now adorns my office wall.

If you like stories of this kind you will revel in *Challenge of Chance,* which is described on the blurb as being "the first book to be written jointly by a Fellow of the Royal Society and a Fellow of the Royal Society of Literature—with a psychologist providing the bridge between the two cultures". The interdisciplinary flavour of the work is certainly striking, but the bridge is rather a shaky one. The book falls uneasily into three separate chunks—the first an account by Sir Alister of an "unsuccessful" mass extra-sensory perception experiment, the second a thirty page dicussion by Harvie of probability theory and how puzzling it all is; the third a long essay by Koestler on some of the strange things that have happened to him, his friends and his correspondents. The majority of the strange things, I hasten to add, are somewhat more marvellous than the CRE 727 example. Hardy's experiment incidentally—a group ESP test in the Caxton Hall—was only unsuccessful in that no evidence of

telepathy was demonstrated. A subsequent and more elaborate search through the data revealed peculiar similarities in the guessing patterns of people seated near to each other in the group test. There are of course a number of possible explanations for such a finding, but Sir Alister considers it to be an example of "the coincidental coming together in time of similar ideas". But what, he asks, do we mean by coincidence anyway? This leads with a bump into the statistician's contribution, where again the inner meaning of the word coincidence is explored. Here the argument appears to be that if you riffle about long enough with sets of numbers you'll discover peculiar sequences in them—but sooner than you ought to have done by chance. Somewhere about here one crosses the transitional zone between statistics and pyramidology and I must confess to not being absolutely clear on occasions on which side of the zone Mr Harvie lies.

One is left in no doubt where Mr Koestler stands however. He doesn't actually use the word 'pyramidology' but like the pyramidologists he clearly believes that man should be very alert to the hidden meanings buried in the depths of what appear to be fairly straightforward objects or events. His section of the book is a compendium of the bizarre and the unlikely—psychic visions, poltergeist-like raps, unusual dreams, chance meetings in strange places and, of course, correspondences of the mystic car number variety. From here he argues that the sheer weight of human testimony to the vast array of oddities of this kind forces one to dispose of the word 'chance' in an explanatory role. Chance itself is a meaningless word which tells us nothing whatsoever about the universe and merely allows us to duck the issue of why such things happen so that we can preserve the creaky yet primary principle of causality. In his final section, ponderously titled "Speculations on Problems Beyond our Present Understanding", he returns to the theme of his previous work, "Roots of Coincidence", arguing that the peculiar phenomena of parapsychology are no longer peculiar in the light of recent discoveries in quantum physics. "Enlightened physicists" he tells us patronisingly, are now willing to concede this. The book is a frank statement of personal opinion and Koestler's segment in particular is highly readable. There's also not much doubt that it will be sweet music to the ears of thousands of readers, including a fair number of scientists who relish the anti-materialistic backlash which Koestler so heartily champions. Curiously, I think it is the parapsychologists, or at least those who have spent their lives attempting to trap the alleged and elusive phenomena of ESP by using the methods of experimental psychology, who will find the book most disquieting. For if there is a central argument which emerges it is that there are facets of the Universe—large facets of it—which will resist and even reject study by the traditional methods of science. This, the book implies, is the principal reason for parapsychologists' signal lack of progress in attempting to 'prove' the existence of ESP. A discouraging argument I would have felt, and if anyone cared to apply it to other areas of science, the consequences for research could be pretty peculiar. Most scientists therefore will find this a glum rather than a heartening book. Perhaps 'glumly entertaining' would be the best way to describe it.

CHRISTOPHER EVANS

Thrips

Thrips: Their Biology, Ecology and Economic Importance. By Trevor Lewis. Pp. xv+349. (Academic: London and New York, November 1973.) £7.80: $22.

THE Thysanoptera are a very interesting and important group of insects which are often ignored, apart from a few economically important species, such as the cereal thrips (*Limnothrips cerealium*) or the onion thrips (*Thrips tabaci*). The literature on this group is therefore very scattered, and this book fulfills a long-standing need by bringing together a large part of this information into one place. It is not a book to be read from cover to cover, it is far too concentrated for that, but small doses from parts of it make fascinating reading for the general entomologist. It is a book that would form a valuable addition to the bookshelf of any entomology or ecology laboratory.

It tries to cover a very wide field indeed, often with rather scant information, as this is all that exists. It includes sections on the general biology of the group, rearing and sampling techniques, ecology and economic importance. This wide coverage, while revealing the enormous gaps in our knowledge, does mean that the available information from both the pure ecological literature and the applied literature is brought together, and this in itself makes the book worth having.

On the whole the book is well presented and the numerous appendices are particularly useful. There is however a number of annoying details. For example, I should have liked to see the plates distributed at relevent places in the text, instead of collected at the end, and why put Fig. 47 (a) on page 135 and Fig. 47 (b) and the legend overleaf on page 136? Comparison would have been very much easier if they had been on facing pages. Errors and inconsistencies inevitably creep into compendia of this sort, but are very few in the present case, for example the corrected catch of thrips at Silwood Park is given on page 205 as "about 18,900 individuals" whereas over the page in Table 24 it is given as 19,900.

The author on the whole has done a good job and this book deserves to be widely used, and will no doubt retain its usefulnes for a long time.

S. MCNEILL

Marine mathematics

The Structure of Marine Ecosystems. By John H. Steele. Pp. x+128. (Blackwell Scientific: Oxford and London, 1974.) £2.75.

THIS "short work" (the author's words) consists of what, otherwise, might have appeared as two or three research papers, preceded by a general discussion about problems of investigating marine ecosystems and followed by some speculations about the impact of man's activities. The core of the book is the author's mathematical simulation of the pelagic ecosystem in the northern North Sea, presented as typifying open sea environments in temperate or sub-arctic waters.

As Dr Steele says, models of this kind reveal the lacunae in one's knowledge and so determine the kinds of research needed in the field and laboratory. In particular, the model helps to assess the validity of simplifications and identify the points at which greater detail is required.

The book serves these objectives admirably. In addition, it provides growing points for debate on topics such as the differences between terrestrial and marine ecosystems, or the significance of ecologists' icons such as diversity, stability, efficiency, food webs and food chains. In conclusion, Dr Steele argues that the most critical parts of marine ecosystems, and the points at which man's activities impinge most seriously, are at the levels of pelagic herbivores and benthic micro-organisms. He suggests that an increasing effort should be devoted to studies of structure and dynamics at these "lower levels" of the system.

The book is amply illustrated with results of Dr Steele's own research; there is an unexpected and probably unnecessary glossary, a short but valuable list of references and a totally inadequate index. It would be a valuable addition to the personal and institutional libraries of ecologists of all kinds, those who read equations as well as those who do not.

R. S. GLOVER

Stratigraphy

The Boreal Lower Cretaceous. Edited by R. Casey and P. F. Rawson. Pp. 448. (Proceedings of a symposium organised by Queen Mary College, London, and the Institute of Geological Sciences, September 1972; *Geological Journal* Special issues no. 5) (Seel House: Liverpool, 1973.) £9.75.

THIS book has been published with the commendable speed and in the attractive form that one has come to expect from the Seel House Press. Owing to its rather specialised nature it will, however, appeal to a more limited audience than the previous geological volumes in this series. The work is basically a well organised and presented compilation of stratigraphic and palaeontological data for a substantial sector of the northern hemisphere by thirty-two authors, mostly English but with a good representation of leading authorities from North America, France, Germany, Denmark, Poland and the Soviet Union. All the articles are in English, with brief summaries in French and German.

It would be both pointless and beyond the scope of a brief review to comment much on individual articles, which range from syntheses of reconnaissance work in large, relatively unexplored territories to reassessments of classic European localities, but I should perhaps single out Casey's substantial contribution on Jurassic–Cretaceous boundary beds in Europe as being especially welcome, because at last he has been persuaded to publish fully, with good illustrations of ammonites, the data on which are based his interesting conclusions on regional correlation. His views on the position of the Jurassic–Cretaceous boundary are well argued, but he has not managed to convince what one might term the Russian school of workers. A consensus on the subject seems as far away as ever. The overall tone of the articles is one of caution and sobriety, so that one greets with raised eyebrows the bold palaeogeographic reconstructions of H. G. Owen, based on the concept of an Earth expanding rapidly at least from the early Mesozoic to the present day. To paraphrase the molecular biologists, he has thereby offended against the 'central dogma' of current earth science, namely plate tectonics. For those with neither the time nor the inclination to plough through the detail, the editors provide a lucid summary of the proceedings in the concluding article.

The outstanding scientific problem concerning the boreal Lower Cretaceous is the cause of the differentiation of the ammonite, belemnite and to a lesser extent other invertebrate faunas from those of the more extensive Tethyan Realm. It is welcome to have many pertinent distributional data brought together in one volume, but disappointing that very few authors have properly got to grips with the problem. All too many seem content to present conventional stratigraphic descriptions and discuss matters of correlation, all very necessary as a framework for environmental interpretation but decidedly unstimulating. Those who do try to account for faunal provinces have little new to offer. Most favour some sort of temperature control, while varying salinity and palaeogeographic configurations are proposed as subordinate factors. What I missed is an awareness of the great strides recently made in ecological understanding, with the introduction of new concepts such as environmental stability which may be highly relevant to the matter in question. Furthermore, nobody has made much progress in gathering quantitative data for particular fossil groups on density, diversity and distributional changes in relation to sedimentary facies, palaeolatitude and so on to test particular hypotheses. I sensed that many of the workers in the field are quite satisfied if they can describe and correlate their strata more accurately than before, and that they regard penetrating enquiries into cause and meaning as somewhat beyond their scope. Where problems of environmental interpretation are dealt with it is often in a somewhat token manner, as though they were of secondary importance. I hope that they are deterred by modesty rather than complacency or unimaginativeness. Perhaps the term used to describe their discipline is unduly inhibiting. In the wake of the dramatic advances being made in other branches of earth science there could be today rather less stratigraphy and more stratology.

A. HALLAM

Thin layer chromatography

Inorganic Chromatographic Analysis. By Jan Michal. Translation Editor: Julian F. Tyson. Pp. x+217. (Van Nostrand Reinhold Series in Analytical Chemistry.) (Van Nostrand: London and New York, March 1974.) £9.

THE original Czechoslovak edition of this book appeared in 1970 and the present English translation is therefore some four or five years out of date. With the dramatic advances in high performance column chromatography which have occurred since 1969 a book dealing almost exclusively with paper and thin-layer techniques (PC and TLC), and making no mention of high performance liquid chromatography or gas chromatography is now rather dated. This would not necessarily be too serious if the author had given an authoritative account of the principles and current equipment, and if he had followed this by a critical appraisal of the application of the techniques in inorganic chemistry. But the treatment of principles is far from adequate: the newcomer would gain little or no insight into the key mechanisms of retention and dispersion of spots or bands in chromatography. He would have to look elsewhere to obtain up-to-date information on equipment; and he would have great difficulty in assimilating the mass of analytical information presented uncritically in the form of a long series of recipes for particular separations, and analyses.

This book cannot be recommended with any enthusiasm, but it may prove useful as a starting point for anyone who wishes to review PC and TLC methods before about 1968, say as a preliminary to devising high performance column methods for some specific separation; but even here its value is prejudiced by the poor subject index and the entire absence of an author index.

J. H. KNOX

Latin American physics

Reaction Dynamics. By F. S. Levin and H. Feshbach. Pp. viii+216. (Documents on Modern Physics.) (Gordon and Breach. New York, London and Paris, July 1973.) £6.65 cloth; £3.50 paper.

I WAS rather disappointed on opening this book to find that is was based on notes taken in some of the lectures of the Latin American School of Physics in 1968. Is it really worthwhile publishing a book in a field of research as rapidly changing as nuclear physics some five years after the event? After reading it I am still not completely convinced.

Levin, whose contribution takes up over three quarters of the book, has helped somewhat by giving a list of supplementary references up to 1972. He makes a survey of many of the "recent" developments in direct nuclear reaction theory, mainly in the description of low energy single particle transfer reactions, such as deuteron stripping. It reads rather like a good review article, with its thorough comparison of theoretical predictions with experiment in over a hundred figures. Most of these predictions were obtained using the distorted wave Born approximation (DWBA), which is the standard way of analysing experimental data, and some space is given to a discussion of the reliability of this as a tool for the extraction of spectroscopic factors. There are ample results on polarisation calculations and the extension of the

DWBA to coupled channel problems. As an alternative theoretical framework, Levin ends with a description of the much more controversial stripping theory of Butler and co-workers, comparing it with both the DWBA and experiment.

The article of Feshbach is very different in character. In just 45 pages it describes some of the features of the theory of nuclear reactions which the author and his collaborators at MIT have developed over the years. Central to this is the interplay between the prompt reactions (the direct reactions discussed by Levin) and the time-delayed ones involving the formation of compound nuclei. By the elimination of the time-delayed components, he shows how an optical model description of the prompt reactions can be formulated, stressing the importance of energy averaging in the scheme. This division into prompt and delayed reactions is of course oversimplistic and Feshbach finishes by considering reactions asociated with characteristic times intermediate between these two extremes, such as doorway states. Unlike the Levin work, these feel much more like lecture notes, intended to give the student a broad overall impression of the field. COLIN WILKIN

Light on molecules

Organic Molecular Photophysics. Vol. 1. Edited by John B. Birks. Pp. xvii+600 (Wiley Monographs in Chemical Physics.) (Wiley-Interscience: London and New York, July 1973.) £13.50.

THIS book is the first of two volumes aiming to provide "comprehensive coverage of the field of organic molecular photophysics" and is intended to be complementary to *Photophysics of Aromatic Molecules* written by Birks. This volume contains ten articles, ranging in subject content from organic dye lasers to exciton interactions in organic solids, which have been written, on the whole, by unquestionable experts in their fields.

The quality of individual articles is extremely high. For example, the treatment of radiationless transitions by Henry and Siebrand presents one of the clearest discussions on this complicated topic. The fluorescence characteristics of aromatic molecules is dealt with thoroughly in two chapters, one by Stockburger, considering low pressure environments and the other, by Offen, dealing with fluorescence and absorption effects at high presures. Triplet–triplet absorptions are discussed from an experimental standpoint by Labhart and Heinzelmann who compile much of the data available up to 1971. There is perhaps some overlap in tabular content between this chapter and "Photophysics of Aromatic Molecules". Dye lasers are dealt with from an experimental and occasional mathematical approach by Snavely; it is a pity that there is little mention in this article on their obvious uses in, for example, high resolution spectroscopy or fluorescence studies of vibronic states. Klopffner deals thoroughly with intermolecular exciplexes. The first chapter on the spectroscopy of pi-electronic states (by Birks) is introductory in nature and those familiar with the author's first work in this series will find the chapter less useful than the rest of the book. Other articles are written on diffusion-controlled rate processes (Alwattar, Lumb and Birks), electron photoejection from aromatic molecules in condensed media (Lesclaux and Joussot-Dubien) and exciton interactions in organic solids (Swenberg and Gaecintov).

I found it difficult to regard the collection of articles as "integrated" since the book reads more like a high quality "Advances in . . . " series. It could be argued that most of the review articles are available elsewhere although not in a single volume. I hope that the high standard of individual articles is maintained in volume 2. This volume would be a very useful addition to the library of individual researchers engaged in photochemistry and photophysics although it would not be essential. M. A. WEST

Atmospheric chemistry

Chemistry of the Lower Atmosphere. Edited by S. I. Rasool. Pp. xii+335. (Plenum: New York and London, 1973.) $26.

THE book is a collection of six chapters on various aspects of atmospheric physics and chemistry by six different authors. Like all such collections it is variable in emphasis, standard and depth of treatment of subject. Because of this it is difficult to decide to what audience the book is aimed.

Dealing with each chapter in turn, the first of about 60 pages entitled "Role of Natural and Anthropogenic Pollutants in Cloud and Precipitation Formation", by Professor H. R. Pruppacher of UCLA, does no more than summarise what can be found in the standard texts on cloud microphysics. Although lip service is paid to the importance of cloud dynamics, that is the air motions leading to cloud condensation, and the interaction between cloud microphysics and dynamics, in fact the chapter is almost exclusively devoted to the microphysics of clouds. There is nothing on the possible modification of precipitation due to anthropogenic pollutants except a brief uncritical reference to some evidence that urban areas experience more precipitation than their adjoining rural environment.

The next chapter, of about 40 pages on "Particulate Matter in the Lower Atmosphere" by Dr R. D. Cadle of NCAR, is a standard review of the main physical and chemical properties of the tropospheric and stratospheric aerosols. The sections on aspects of the atmospheric sulphur cycle and the scavenging processes for particulates in the troposphere duplicate more extensive treatments of these aspects in later chapters.

The third chapter is on "Removal Processes of Gaseous and Particulate Pollutants" by Dr G. M. Hidy, and is perhaps the best chapter in the book. It contains a scholarly and comprehensive treatment of the atmospheric scavenging processes.

"The Global Sulphur Cycle" is the title of the next section (24 pages) by Dr J. P. Friend. It summarises effectively the current state of knowledge of sources, sinks and reservoirs of sulphur. It is nice to see an author emphasising the limitations of his treatment.

The next contribution of 46 pages is perhaps the one closest to the central theme of the book, in that it deals with "The Chemical basis for Climate Change". The authors are Drs Schneider and Kellogg of NCAR. In it are described the qualitative arguments used to ascribe possible casual relationships between changes in atmospheric composition and climate. It also gives the reader some feel for the vast problems involved in any worthwhile quantitative modelling of climatic change.

The last chapter of 79 pages, by Dr C. D. Keeling, is devoted to the carbon dioxide cycle. It could be argued that the mathematical detail contained in this chapter is out of proportion to that in the rest of the book, but nevertheless it gives a very real insight into the various interacting factors concerned with the atmosphere, the oceans and the biosphere which control the concentration of CO_2 in our atmosphere.

In summary, the book has little to offer those workers actively engaged in the fields of atmospheric chemistry and meteorology. But at a time when more and more scientists outside the fields of atmospheric physics and chemistry are taking an interest in pollution matters, this book provides a useful introduction to some aspects of the subject. P. GOLDSMITH

Erratum

The price given for *Comparative Vertebrate Morphology* by Douglas and Molly Webster (reviewed in *Nature*, **249**, 866; 1974) was incorrect; it should have been $14.95 and £7.

science on television

One giant bore

by John Gribbin

ON JULY 20, just five years to the day since Neil Armstrong and Edwin Aldrin stepped on to the Moon, the BBC broadcast, with much huffing and puffing from its publicity machine, "Moonwalk One"; ". . . a NASA-commissioned documentary which records the moon landing adventure in minute and breathtaking detail", to quote from the *Radio Times*.

Minute the detail may have been, but the programme was hardly breathtaking, except insofar as the encouragement of yawning could be said to constitute a breathtaking spectacle. This was an opportunity missed on the grand scale; a film which should be preserved as a guide to producers in how not to present exciting science on television.

Shots of Saturn rockets taking off are now something of a cliché, but still sufficiently spectacular to justify inclusion in such a supposedly definitive feature. But there was little further material with a comparable visual impact, in spite of the existence of miles of such film in the NASA archives. The reason for this seems to have been a desire on the part of the makers to use only genuine Apollo 11 material, in line with the programme's title.

That would be laudable enough if the material was any good, but it simply is not. To me, the most dramatic thing about the first Moon landing was that it was covered live on TV around the world. As far as I know, this was just about the only aspect of the mission not predicted by the science fiction writers.

But in retrospect those first pictures were not at all good. Since Apollo 11, we have become even more blasé about the TV coverage of Apollo, and used to pictures which are not just shaky grey blurs labelled "Live from the Moon", but rather in full colour and coming from a camera which is controlled from Earth so that we can watch the astronauts at work.

The answer, surely, would have been to produce a documentary not just about Apollo 11 but about the whole Moon adventure, using material from the later missions which is so ideally suited to the TV medium. As it was, whether through the paucity of good Apollo 11 material or as policy, "Moonwalk One" fell back on long, boring sections of padding. Shots of row upon row of the 'backroom boys' at Houston no doubt brought a warm glow to the hearts of their Moms and kids back home, but told us little of the excitement and importance of the mission. And views of good American citizens flocking lemming like to the beach to watch the launch will no doubt provide useful information for sociologists studying the phenomenon of how dramatically public interest in the Apollo programme waned after the first mission. But that, surely, was not what the programme was all about.

Even the genuinely exciting parts of the programme—perhaps one sixth of its ninety-minute length—were marred by obtrusive and inappropriate background music in the sub-2001 tradition, and the few graphic aids used were not in the same league as the famous 'simulation' which filled in the gaps when the live Apollo broadcasts were being made. Some heretics, indeed, have been known to argue that the simulation was, visually, the best part of those missions; they have a point, but perhaps those of them who watched "Moonwalk One" were mollified by the segment of old Buck Rogers' film clips. These provided the dramatic highlight of the programme, which declined monotonically in interest for its final hour.

The tone of the whole proceedings is perhaps best summed up by noting that the *Radio Times* even failed to quote Armstrong's famous and carefully rehearsed *ad lib* correctly: "One small step for man, one big step for mankind", they said. None out of ten for effort; whoever wrote that should be encouraged to take a giant leap.

But faith in the American way of life and a genuinely breathtaking spectacle was provided to soothe the heated brow within an hour of the end of "Moonwalk One". "Sergeant Bilko", starring Phil Silvers—now, that *was* worth staying up late for.

obituary

L. C. Dunn

LESLIE C. DUNN, one of America's oldest and most influential geneticists, died unexpectedly on March 19, 1974 still active in a scientific career which spanned more than sixty years, encompassing almost all of the development of modern genetics. His early work established some of the first cases of linkage in mammals, and he was the first to demonstrate similar degrees of linkage between what seemed to be the same kinds of mutations in two distinct species, mice and rats. This led him to recognise that Mendelian analysis could yield clues to genetic homologies and relationships among species, and thus be applicable to understanding the evolution of natural populations, and formed the basis of his life-long interest in what was to become population genetics. His chief work, however, continued to be genetic analysis, and the major efforts of his laboratory centred on the analysis, by both genetical and embryological methods, of mutations expressed in early stages of embryonic life. The greatest part of this work concerned one extensive series of mutations at a complex region (T) in the mouse, where he and his colleagues analysed a number of mutations which identified genetic factors essential to early embryonic development. After the elements of this system had been identified in laboratory mice, his interest in natural populations led him to examine feral groups of *Mus* for the presence of these mutations. Most of such populations in North America and Europe proved to be polymorphic for variant genes, usually embryonic lethals or semilethals, belonging to the T series. In searching for explanations of how lethal genes could be main-

tained at high frequencies in natural populations, he found that all alleles at this locus found in the wild were transmitted by heterozygous males to 90–99% of their offspring. This evolutionary force—gametic selection—was thus more powerful than the force of natural selection which would eliminate homozygous embryos.

In addition to these purely scientific activities, Professor Dunn was convinced that relations between nations and cultures could be improved by using scientific collaboration as a bridge. As an army officer returning from Europe in 1919, he helped to provide agencies in the Soviet Union with scientific and technical literature useful in the new state which arose from the revolution of October, 1917. He visited the Soviet Union in 1927 at the time of the 10th Anniversary of the Revolution and thereafter remained in close contact with the active school of genetics there. During the Second World War he was President of the American–Soviet Science Society which undertook the exchange of scientific and technical publications. The same interest in international scientific collaboration was responsible for his part in organising facilities in the USA for enabling scholars displaced by the Nazi takeover of 1933 to continue their work in American universities.

What began then as the Emergency Committee for German Scholars became, as fascism spread, The Emergency Committee in Aid of Displaced Scholars, and he served as a member of its Executive Committee from 1933 until its dissolution in 1947.

Always interested in questions of race, he began in 1933 actively to apply biological ideas to social and political questions, and to speak and write about heredity in relation to racial prejudice in ways designed for the general public. This led, in collaboration with his friend and colleague, Th. Dobzhansky, to the publication in 1946 of *Heredity, Race and Society,* a book which has now appeared in almost all languages, and subsequently in 1951 to a Unesco Report, *Race and Biology;* both of these were fundamental in establishing the then novel view of human races as populations differing in the relative frequencies of genes shared by most populations.

These activities were an expression not only of his interest in science as an instrument of international education, but of his view that, to be effective, efforts of this sort had eventually to achieve a political forum. Other expressions of the latter view can be found in advisory and consultative work beginning in 1940 in aid of efforts to devise a means for the support of science by the United States Government. He was involved in early drafts of legislation which are predecessors of that which finally brought about the creation of the United States National Science Foundation in 1950.

Professor Dunn received the ScD from Harvard University in 1920, and after a time at the Agricultural Experiment Station in Storrs, Connecticut, joined the Faculty of Columbia University as Professor of Zoology in 1928. At the time of his death he was Emeritus Professor and Senior Research Scientist at Columbia. He was a member of the National Academy of Sciences (USA), the American Academy of Arts and Sciences, the American Philosophical Society, the Norwegian Academy of Arts and Sciences, and the Academia Patavnia; he had been President of the Genetics Society of America, the American Society of Naturalists, and the American Society of Human Genetics. He also served on many editorial boards, and was for some years managing editor of *Genetics,* and later of the *American Naturalist,* the oldest biological journal in the United States. In addition to *Heredity, Race and Society,* his books include *Principles of Genetics, Heredity and Evolution in Human Populations,* and *A Short History of Genetics.*

His associates in all his various endeavours will remember him for his stubborn integrity, his sure and immediate grasp of the essentials of any problem or situation, and his complete freedom from pretence or self importance of any kind. Many of us who knew him will continue to measure our own success by the degree to which we live up to the standards which he set.

Announcements

Awards

The **Barclay Prize** of the **British Institute of Radiology** has been awarded to **Godfrey Hounsfield** and **James Ambrose.**

Corrigendum

In the article "Batesian mimicry without distastefulness" by D. O. Gibson (*Nature,* **250,** 77; 1974) the quotation in the penultimate sentence should read . . . in the population 'the selective disadvantage of being common begins to outweigh the advantage of being conspicuous'[4] and predation will . . .

Reports and Publications

Great Britain

British Antarctic Survey. Scientific Reports, No. 66: Crustal Structure of the South Shetland Islands and Bransfield Strait. By W. A. Ashcroft. Pp. 43. (London: British Antarctic Survey, Natural Environment Research Council, 1972.) £2.25 net. [136

The Grassland Research Institute. Annual Report for 1973. Pp. 88. (Hurley, Maidenhead: Grassland Research Institute, 1974.) £1.50. [176

The Kent Incorporated Society for Promoting Experiments in Horticulture. East Malling Research Station Report for 1973 (1st October, 1972 to 30th September, 1973.) Pp. xii + 240. (East Malling, Maidstone: East Malling Research Station, 1974.) £1.50; $5. [176

University of Oxford. Tenth Annual Report of the Delegates of the Science Area for the year ending 31st July 1973. Pp. 149. (Supplement No. 4, to the *University Gazette,* April 1974.) (Oxford: The University, 1974.) £1. [176

Medical Research in too Important to the left to the Researchers. By Professor W. S. Peart. (Lecture given at the Royal Institution, London, on 18th October, 1973, to mark the 25th Anniversary of the Foundation of the Glaxo Volume). Pp. 16. (Greenford, Middx.: Glaxo Laboratories, 1974.) [176

The British Institute of Radiology. Annual Report 1973/1974. Pp. 10. (London: The British Institute of Radiology, 1974.) [196

Research Fields in Physics at United Kingdom Universities and Polytechnics. Pp. xv + 343. (London and Bristol: The Institute of Physics, 1974.) £7. $17.50. [196

First Report of the Advisory Board for the Research Councils. Pp. 28. (Cmnd. 5633). (London: HMSO, 1974.) 20p net. [206

University Grants Committee. Statistics of Education 1971. Vol. 6: Universities. (Department of Education and Science Series.) Pp. xxxvi + 149. (London: HMSO, 1974.) £2.90 net. [206

Building Research Establishment Digest No. 166: European Product-Approval Procedures—1. Pp. 4. (London: HMSO, 1974.) 5p. [206

Report on Cetacea Stranded on the British Coasts from 1948 to 1966. By F. C. Fraser. Pp. 65 + 9 maps. (London: British Museum (Natural History), 1974.) £3. [206

Research and Development in France. Pp. 36. (London: Ambassade de France, Service Scientifique, 1974.) [216

Other countries

World Health Organization—International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 4: Some Aromatic Amines, Hydrazine and Related Substances, N-nitroso Compounds and Miscellaneous Alkylating Agents. Pp. 286. Sw. fr. 18. Vol. 5: Some Organochlroine Pesticides. Pp. 241. Sw. fr. 18. (Geneva: WHO; London: HMSO, 1974.) [186

Smithsonian Contributions to Botany, No. 13: Swollen-Thorn Acacias of Central America. By Daniel H. Janzen. Pp. 131. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) $2.35. [186

Smithsonian Studies in History and Technology, No. 24: Wheels and Wheeling—The Smithsonian Cycle Collection. By Smith Hempstone Oliver and Donald H. Berkebile. Pp. v + 104. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) $1.90. [186

Onchocerciasis: Symptomatology, Pathology, Diagnosis. Edited by A. A. Buck. Pp. 80. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 12. [186

Guidelines for the Laboratory Diagnosis of Cholera. Prepared by the WHO Bacterial Diseases Unit. Pp. 23. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 5. [186

Alfred P. Sloan Foundation. Report for 1973. Pp. viii + 79. (New York: Alfred P. Sloan Foundation 630 Fifth Avenue, 1974.) [186

Smithsonian Contributions to Zoology, No. 156: A Revision of North American *Capitophorus* Van der Goot and *Pleotrichophorus* Borner (Homoptera: Aphididae). By Leonila Alzate Corpuz-Raros and Edwin F. Cook. Pp. iv + 143. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) $2.25. [196

World Meteorological Organization. Technical Note No. 133: An Introduction to Agrotopoclimatology. By L. B. MacHattie and F. Schnelle. Pp. xii + 131. (Geneva: WMO, 1974.) [196

Office de la Recherche Scientifique et Technique Outre-Mer. Collection Travaux et Documents de l'ORSTOM, No. 28: Kinkala—Etude d'Un Centre Urbain Secondaire au Congo-Brazzaville. Par Alaun Auger. Pp. 132. (Paris et Bondy: ORSTOM, 1973.) 42 francs. [196

Australian Academy of Science. Science and Industry Forum. Report No. 8: The Future Education of Scientists. (Papers delivered at the 13th Forum Meeting, 11 February 1973.) Pp. 53. (Canberra: Australian Academy of Sciences, 1973.) [206

World Health Organization. Technical Report Series, No. 546: Assessment of the Carcinogenicity and Mutagenicity of Chemicals—Report of a WHO Scientific Group. Pp. 19. Sw. fr. 4. No. 548: Planning and Organization of Geriatric Services—Report of a WHO Expert Committee. Pp. 46. Sw. fr. 5. (Genewa: WHO—London: HMSO, 1974.) [216

Australian Academy of Science — Descriptive Brochure. Pp. 27. (Canberra: Australian Academy of Science, 1974.) [216